Institut Pasteur
du Cambodge

ANNUAL REPORT 2022

Institut Pasteur du Cambodge

Pasteur Network

Table of Contents

1. List of Acronyms

AAS	Atomic Absorption Spectrophotometer
ABR	Antibiotic Resistance
ACIP	Action Concertée Inter-Pasteurienne (Institut Pasteur)
ACT	Artemisinin Combination-based Resistance
ADE	Antibody-Dependent Enhancement
AFD	Agence Française pour le Développement (French Development Agency)
AFRIMS	Armed Forces Research Institute for Medical Sciences (USA)
AIRD	Inter-établissements de recherche pour le développement International
AHF	AIDS Health Care Foundation
AVSF	Agronomes et Vétérinaires Sans Frontières
AIV	Avian Influenza Viruses
AMP	Antimicrobial Peptides
AMR	Antimicrobial Resistance
ANRS-MIE	Agence Nationale de Recherche sur le SIDA, les Hépatites et les maladies infectieuses et émergentes (French National Agency for AIDS and Hepatitis Research)
AQ	Amodiaquine
AQ-R	Amodiaquine Resistant(ce)
ART-R	Artemisinin resistant
ARCAHE	Antibiotic resistance at the Human/Animal/Environment interface in a “One Health” Approach in Cambodia – FSPI project
ARCIMED	Antimicrobial Resistance Circulation along the Mekong and its Delta
AS	Arsenic
ASAQ	Artesunate amodiaquine
ASIDE	Alerting and Surveillance for Infectious Diseases Epidemics
AST	Antibiotic Susceptibility Testing
AVIESAN	Alliance Nationale pour les Sciences de la Vie et de la Santé, France (French Alliance for Life Sciences and Health)
Beta-CoVs	Beta Coronaviruses
BP	Burkholderia Pseudomallei
BSL	Biosafety level
BIRDY	Bacterial Infections and Antibiotic-Resistant Diseases among Young Children in Low Income Countries
BMGF	Bill and Melinda Gates Foundation
BOD	Burden of Disease
BTRP	Biological ThreatReduction Program
CANARIES	Consortium of Animal Networks to Assess Risk of Emerging Infectious Diseases through Enhanced Surveillance
CARAMAL	Community Access to Rectal Artesunate for Malaria
CCBR	Cambodian Committee for TB Research
C-CDC	Cambodian Center for Disease Control and Prevention
CEO	Chief Executive Officer
CFO	Chief Financial Officer
CD	Cadmium
CEG	Community Epidemiology Group

CIRAD	Centre de Coopération Internationale en Recherche Agronomique Pour le Développement, (French Agricultural Research and International Cooperation Organization)
CEIRR	Centers for Excellence in Influenza Research and Response
CENAT	National Center for Tuberculosis and Leprosy Control (Cambodia)
CERFIG	Centre de Recherche et de Formation en Infectiologie de Guinée
CHIKV	Chikungunya Virus
CIN	Cervical Intraepithelial Neoplasia
CLPD	Chronic Lymphoproliferative Disorder
CNM	National Center for Parasitology, Entomology, and Malaria Control (Cambodia)
CNRS	Centre National de la Recherche Scientifique
COFRAC	French Accreditation Committee (Comité Français d'Accréditation)
COVID-19	Coronavirus Disease 2019
CPC	Centre Pasteur du Cameroun
CPE	Carbapenemase Producing Enterobacteria
CREID	Centers for Research in Emerging Infectious Diseases
CRG	Clinical Research Group
CSS	Cross Sectional Survey
CVRP	Comprehensive Viral Research Panel
CWRU	Case Western Reserve University (USA)
DAA	Direct Acting Antiviral Therapy
DARPA	Defense Advanced Research Projects Agency
DBS	Dried Blood Spot
DENV	Dengue Viruses
DF	Dengue Fever
DHA-PPQ	Dihydroartemisinin Piperaquine
DHF	Dengue Hemorrhagic Fever
DNA	Deoxyribonucleic Acid
DRC	Democratic Republic of Congo
DSS	Dengue Shock Syndrome
DTG	Dolutegravir
DTRA	Defense Threat Reduction Agency (USA)
DVI	Dengue Vaccine Initiative
EC	Escherichia coli
EID	Emerging Infectious Disease
ENTOMO	Medical and Veterinary Entomology Unit of Institut Pasteur du Cambodge
EPH	Epidemiology and Public Health Unit of Institut Pasteur du Cambodge
EOC	Emergency Operational Center
ERIG	Equine Rabies Immunoglobulins
ES	Environmental Sampling
ESBL-E	ESBL-producing Enterobacteriaceae
FAO	Food and Agriculture Organization of the United Nations
FAVN	Fluorescent Antibody Virus Neutralization
FCI	Flow Cytometric Immunophenotyping
FE	Lead
FG	Forest Goers
FLDs	Fragmented and Loop Primer Ligated dsRNA Sequencing

FQ-Rs	Resistant (ce) to Fluoroquinolones
FRNT	Foci Reduction Neutralization Test
FSPI	Solidarity Fund for Innovative Projects- France
GCRF	Global Challenges Research Fund
GDAPH	Department within the Cambodian Ministry for Agriculture, Forestry and Fisheries
GF	Global Fund to Fight AIDS, TB and Malaria
GIS	Geospatial Information System
GISAID	Global Initiative on Sharing Avian Influenza Data
GISRS	Global Influenza Surveillance and Response System
GLASS	Global Antimicrobial Resistance Surveillance System
GMS	Greater Mekong Subregion
GO	Grant Office
GPS/GSM	Global Positioning System and Global System for Mobile Communications
HBV	Hepatitis B Virus
HC	Health Center
Hcase	High-level Cephalosporinase
HCV	Hepatitis C Virus
HDN	Human Baited Double Net Traps
HEPAR	Hepatitis E and ARENAvirus Project
HEV	Hepatitis E Virus
HFMD	Hand-Foot-and-Mouth Disease
HITS	High Impact Transformative Science
HIV	Human Immunodeficiency Virus
HSeQM	Health, Safety, Environment, Quality, Metrology
HS-RDT	High Sensitivity Rapid Diagnostic Test
HLC	Human-land Catching Barrier Screens
HLA	Human Leukocyte Antigen
IAS	International Accreditation Service
IAV	Swine Influenza A Virus
ICEMR	International Center of Excellence in Malaria Research
ICP-OES	Inductively Coupled Plasma - Optical Emission Spectrometry
IEC	Information, Education and Communication
IF	Impact Factor
ILI	Influenza-like Illnesses
IMMUNO	Immunology Unit, IPC
INF	Interferon
INRAE	Institut National de la Recherche pour l'Agriculture, l'Alimentation et l'Environnement
INSERM	Institut National de la Santé et de la Recherche Médicale (France)
INSTI	Integrase Strand Transfer Inhibitor
IP	Institut Pasteur (Pasteur Institute)
IPC	Institut Pasteur du Cambodge
IPP	Institut Pasteur de Paris
IPIN	Institut Pasteur International Network
IPL	Institut Pasteur du Laos
IRD	Institut de Recherche pour le Développement (Institute for Research and Development-France)
IRIS	Immune Reconstitution Inflammatory Syndrome

ITC	Institute of Technology (Cambodia)
IVI	International Vaccine Initiative
IZSAM	Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise
JEV	Japanese Encephalitis Virus
KBH	Kantha Bopha Hospital
KP	Klebsiella pneumoniae
LAM	Live Animal Market
LBM	Live Bird Market
LEFS	Laboratory for Environment and Food Safety Laboratory (Service open to public)
LIMS	Laboratory Information Management System
LORV	Loei River Virus
LMICs	Low-middle Income Countries
LMI-DRISA	International Joint Laboratory “Drug Resistance in Southeast Asia” (IRD)
LSHTM	London School of Hygiene and Tropical Medicine
LTBI	Latent Tuberculosis Infection
MBL	Medical Biology Laboratory (open to public)
MAFF	Ministry of Agriculture, Forestry, and Fisheries (Cambodia)
MALDI-TOF	MS Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry
MD	Master Degree
MDR	Multidrug Resistant(ce)
MEAE	Ministère de l’Europe et des Affaires Etrangères (French Ministry for European and Foreign Affairs)
MEAE	Ministère de l'Europe et des Affaires étrangères
MEF	Ministry of Economy and Finance (Cambodia)
MERS	Middle East Respiratory Syndrome
MESRI	Ministère de l’Enseignement Supérieur, de la Recherche, et de l’Innovation. (French Ministry for Higher Education, Research and Innovation)
MOEYS	Ministry of Education, Youth and Sports (Cambodia)
MIE	Maladie Infectieurse Émergente (Emerging Infectious Disease)
MiVEGEC	Maladies Infectieuses et Vecteurs Écologie, Génétiques, Évolution, et Contrôle (IRD, France)
MinION	DNA sequencing using Oxford Nanopore Technologies
miRNA	Cell-free circulating microRNA biomarkers
MLST	Multilocus Sequence Typing
MOE	Ministry of Environment
MOA	Memorandum of Agreement
MOH	Ministry of Health (Cambodia)
MOOC	Massive Open Online Course
MORU	Mahidol Oxford Tropical Medicine Research Unit
MoU	Memorandum of Understanding
MMV	Medicines for Malaria Venture
MP-AES	Microwave Plasma Atomic Emission Spectroscopy
MPA	Molecular Multiplex Assays
MR	Malaria Research Unit
MSP	Main Spectra Projection
MTB	Mycobacterium Tuberculosis
MRSA	Methicillin-resistant Staphylococcus aureus

MSAT	Mass Screening and Treatment
MSM	Men who Have Sex with Men
MTCT	Mother to Child Transmission of HIV
MTRU	Malaria Translational Research Unit
nAb	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 (Cambodia)
NGO	Non-Governmental Organization
NIAID	The National Institute of Allergy and Infectious Diseases (US)
NIC	National Influenza Center
NIH	National Institutes of Health (USA)
NIP	National Immunization Program
NIPH	National Institute for Public Health (Cambodia)
NABM	Nomenclature of Medical Biology Acts (NABM)
NGS	Next Generation Sequencing
NMCH	National Maternal and Child Health Center
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NPHL	National Public Health Laboratory
NSSF	National Social Security Fund
NUCS	Northeastern University College of Science
NUS	National University of Singapore
OHARAT	One Health Anthropological approach to RAT
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
PEP	Post Exposure Prophylaxis
PHC	Primary Health Care
PhD	Doctor of Philosophy (degree)
PHD	Public Health Department
PHEIC	Public Health Emergency of International Concern
PIB-Net	Pasteur International Bioresources Network
PICREID	Pasteur Institute – Center for Research for Emerging Infectious Diseases
PLHIV	People living with HIV
PMI	President’s Malaria Initiative (USA)
PN	Pasteur Network
POC	Point of Care
POCTs	Point of Care Test(s)
PPR	Programme Prioritaire de Recherche
PREDICT	Pandemic Preparedness for Global Health
PREEMPT	Preventing Emerging Pathogenic Threats
PrEP	Pre-exposure Prophylaxis
PREZODE	Preventing Zoonotic Disease Emergence
PRR	Platforme Régionale de Recherche
PSI	Population Services International
PTR	Programme Transversal de Recherche (funding mechanism - Institut Pasteur Paris)

PVDBP	P. vivax Duffy Binding Protein
QA/QC	Quality Assurance/Quality Control
QFT	Quantiferon
QWArS	Qualifying the Workforce for AMR Surveillance in Africa and Asia
RABV	Rabies Virus
RAI3	Regional Artemisinin Resistance Initiative
RAS	Resistance-Associated Substitution
RBC	Red Blood Cell
RDT	Rapid Diagnostic Test
RGC	Royal Government of Cambodia
RNA	Ribonucleic Acid
RUA	Royal University of Agriculture (Cambodia)
RUPP	Royal University of Phnom Penh (Cambodia)
RPC	Rabies Prevention Center
RSV	Respiratory syncytial virus
RVNA	Rabies virus-neutralizing antibodies
SARI	Severe Acute Respiratory Illness
SAB	Scientific Advisory Board
SADS	Swine Acute Diarrhea Syndrome Coronavirus
SARS-CoV-2	Severe Acute Respiratory Illness, COVID-19- 2
SEA	Southeast Asia
SeOV	Seoul Ortho Hantavirus
SHC	Social Health Science Clinic
SHCH	Sihanouk Hospital Center of Hope (Cambodia)
SIV	Swine Influenza Virus
SMRU	Shoklo Malaria Research Unit
SNP	Single Nucleotide Polymorphism
SOP	Standard Operating Procedures
STI	Sexually Transmitted Infections
SVR	Sustained Virological Response
TB	Tuberculosis
TBD	Tick-borne Disease
TBT	Tuberculosis Preventive Treatment
TEMVS	Viral Metagenomic Sequencing
TGW	Transgender Women
THAIV	Thailand Hantavirus
TLR	Toll-like Receptors
TPT	Tuberculosis Preventive Treatment
TWG	Technical Working Group
UGA	University of Georgia (USA)
UHS	University of Health Sciences (Cambodia)
UMD	University of Maryland
UMR	Unité Mixte de Recherche (joint research units) France
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNICEF	United Nations International Children's Emergency Fund
USAID	United States Agency for International Development
UP	University of Puthisastra (Cambodia)

UPS	Université Paris Saclay
US CDC	US-Centers for Disease Control and Prevention
UTI	Urinary Tract Infections
VC	Vaccination Center
VEG	Veterinary Epidemiology Group
VACC	International Vaccination Center (service open to public)
VCCT	Voluntary Confidential Counselling and Testing for HIV
VIRO	Virology Unit, Institut Pasteur du Cambodge
VL	Viral Load
VOC	Variants of Concern
WCS	Wildlife Conservation Society
WEHI	Walter and Elizabeth Hall Institute (Australia)
WENV	Wēnzhōu Virus
WHO	World Health Organization
WHOCC	WHO Collaborating Center on Influenza- Melbourne Australia
WNV	West Nile Virus
ZIKV	Zika Virus

2. The IPC in 2022 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 activities of the Institute in 2022, a year of gradual return to a normal situation after many months marked by the COVID-19 pandemic in the world and in Cambodia.
Our Institute remains very attractive with, as of 31 December 2022, 293 people of 14 nationalities working there. The scientific activities are carried out by more than 35 scientists, each holding at least a PhD, sometimes coupled with a doctorate degree in medicine, veterinary medicine, or pharmacy.

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

In 2022, to strengthen the interaction between research units, 3 working groups have been created or reorganized, for antimicrobial resistance, rabies and arboviruses. A notable development this year regards ticks, with two projects focused on (i) tick diversity and their endofauna to bring light on tick-borne diseases and their transmission in rural Cambodia (NOSI-TICK fundes by NIH), and (ii) the development a determination key for tick species in all Southeast Asian countries (SEA TICKEY). This latter project will initiate and develop veterinary entomology in Cambodia, with the Royal University of Agriculture and the Ministry of Agriculture as our main partners.

Research activities carried out in 2022 comprise the publication of 53 articles by scientists affiliated to IPC, and appearances in peer-reviewed international journals with impact factors (IF) higher than 0. Among these appearances, 19 were as first or last author, and 34 had an IF greater than or equal to 4.

The public health activities carried out by the 3 IPC's WHO reference centres included: continued monitoring of human and avian influenza viruses and SARS-COV-2 with, in particular, the monitoring of SARS-CoV2 variants which appeared successively in 2022, and the investigation of a human case of avian influenza (H9N2) alongside—and in support of—the MoH teams. Post-exposure rabies management activities increased by 43 % in 2022 as compared to 2021, with the management of 63,041 patients in our 3 rabies prevention centers. The rabies risk remains high in Cambodia. Indeed, 74% of the animal tests for rabies virus at the Virology Unit were positive. In 2022, discussions were also initiated between IPC's Management and the Directors of Kampong Cham Province's Health

Department and of Kampong Cham's Provincial Hospital, to rebuild the Rabies Center within the hospital compound, and to make it more functional and accessible.

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 Sciences (UHS) in Phnom Penh, and welcomes many students for internships and practical experiences. In 2022, 73 students interned at IPC. This is higher than the 55 hosted in 2021, which number was limited due to the continuing COVID-19 epidemic. Among the 2022 students, 59 were Cambodian nationals, while the others were either French, Indonesian, Thai or Columbian.

As part of the training of these young scientists, a doctor from the Clinical Research Group, currently pursuing a Master's degree at the London School of Hygiene & Tropical Medecine, joined the body of scientists from the Pasteur Network, bringing to 9 the number of "Cambodian statutory scientists" at the IPC.
Finally, an aid policy for young Cambodians has been put in place so that they can follow their PhD at the IPC (scholarship for non-IPC students, payment of their salary during their PhD for people from the iPC). Thus, in the last quarter, the IPC housed 9 PhD students, including 7 Cambodian nationals.

Services activities increased strongly in 2022, compared to 2021: (i) + 38.6 % for the Medical Biology Laboratory's activities (8.3 million of "B"), (ii) + 11 % for the total number of tests performed by the Laboratory of Environment and Food Safety, which was accredited by the International Accreditation Service (IAS) in Seprember 2022, and (iii) + 82 % for the total number of injections (including vaccinations and immunoglobulins) at the International Vaccination Center.

Highlights of 2022

Human Resources

- Dr Jean Popovici succeeded Dr Witkowski as Head of the Malaria Research Unit in September 2022.
- Dr Nathalie De Rakanière took over the leadership of the "Clinical Research Group" within the Epidemiology and Public Health Unit in November 2022, replacing Dr Laurence Borand.
- Dr Dim Bunnet, from the "Clinical Research Group" joined the body of "Pasteur Network Scientists" as "Research Fellow" in August 2022.
- Dr Ly Sowath was promoted to the rank of "Research Director" in the "Pasteur Network Scientists" body.

SARS-CoV 2 Surveillance

PCR Testing

Due to the easing and end of the COVID-19 epidemic during the year 2022, and to the end of the MoH's reimbursement of tests carried out by the IPC for the benefit of the various MoH structures in April 2022, the number of PCR tests performed at the IPC decreased. As of 31 December 2022, 942,703 RT-PCR tests had been performed since the start of the pandemic, i.e. 53,166 for the whole of 2022 (889,537 as of 31 December 2021).

SARS-CoV Monitoring: Omicron and other VoC

Omicron subvariant groups BA.1, BA.2, BA.4, and BA.5 were detected in Cambodia in 2022. 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 around 95 % of Omicron sequences positive for this subvariant after week 8, 2022. Throughout 2022, the Virology Unit monitored the emergence of the different variants and regularly informed the health authorities (MoH, CDC-MoH, etc.).

Surveillance and Investigation of the Avian Flu Virus: Confirmation of A/H9N2 Influenza in a 1-Year-Old Female Patient in March 2022

One sample was collected at Angkor Children's Hospital from a 1 year, 1 month old female individual ILI case from Trapaing Roun village, Roesei Lok commune, Chi Kreng district, Siem Reap province on 4 March 2022. The individual had a slight fever (>38°C), cough, a sore throat, a runny nose, and difficulty breathing. A sample was tested at NIPH on 5 March 2022, and influenza A, subtype A/H9N2 was identified on 8 March 2022. The sample was then transferred to *Institut Pasteur du Cambodge*'s (IPC), Virology Unit on 9 March 2022 and was tested for Influenza type A and B, as well as for hemagglutinin (HA) subtypes of human seasonal A/pdmH1 and A/H3, and avian subtypes A/H5. Furthermore, A/H9. RNA was extracted from the sample and was analyzed by standard RT-qPCR assays. Virological results are shown in the table below. The sample was confirmed positive for Influenza A(H9) and negative for all other tests. Re-extraction and retesting of the sample validated those results.

The report was sent to the MoH on 10 March.

Quality Assurance and Accreditation

The LEFS was accredited by the International Accreditation Service (IAS) on September 2022 for food microbiology analysis under the accreditation number TL-1056, according to the ISO/IEC 17025:2017 standard. The LEFS is our third accredited entity, after the MBL and the Metrology Laboratory within HSeQM.

The Medical Biology Laboratory (MBL) was successful in having its ISO 15189 accreditation confirmed by the French Accreditation Committee (COFRAC) for the fifth time, with a scope of accreditation covering over 60 % of its analysis work.

Improving Security and Service Quality for Patients: Infrastructure Works

With support from the British Embassy in Cambodia, several important improvements were made to the physical infrastructure at the IPC entrance/reception. These improvements had multiple objectives:

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

Work was completed at the end of January 2022.

BSL-3 Laboratory Maintenance

In May, the BSL-3 laboratory successfully underwent its first post-COVID-19 maintenance and certification. This maintenance was accompanied by a technical audit carried out by the *Institut Pasteur*. In November, a complete renovation of the air treatment ducts was done.

Missions and Visits to IPC

With the end of the COVID-19 epidemic, visits and missions have resumed at the IPC. Most notable among them was the visit of Prof. Stewart Cole, Director General of the Institut Pasteur, who made the trip on the occasion of the May 23 Liaison Council. He had the honor to be granted an Audience with his majesty Norodom Sihamoni, King of Cambodia, in testimony of the Institute's importance in Cambodia. His Majesty expressed Cambodia's pride in hosting such a structure and expressed his warmest thanks for the work accomplished over almost 70 years for the benefit of the Cambodian population.

Royal Audience of Prof. Stewart Cole (Institut Pasteur de Paris) in the presence of Mr Jacques Pellet (French Ambassador to Cambodia), Prof. André Spiegel (Director of the IPC) and Dr Ly Sowath (Deputy Director of the IPC)

On 16 November, the Institut Pasteur du Cambodge (IPC) had the pleasure of welcoming a delegation from the French National Research Institute for Sustainable Development (IRD) led by Mrs Valérie VERDIER, its Chairwoman of the Board and CEO.

Study on the IPC Campus Renovation: "IPC-2030 Project"

The Institute remains very attractive, and its numbers are growing every year. One of the factors limiting the IPC's development is the narrowness of its technical premises and of the campus in general. An evaluation was undertaken in 2022 by the Bloom Architecture firm, which carried out the modification of the IPC's accessways. The firm's conclusions confirmed that the buildings original foundations and the evolution of urban planning do not allow the laboratories to be raised by either one or two floors. In a long-term vision, it will therefore be necessary to completely rebuild one or more of our buildings.

More specific studies will be continued in 2023 in order to determine the needs of our operational staff, and to develop a timetable and a first draft. This ambitious "IPC 2030" project will be presented to the Ministry of Health, whose opinions, authorizations and support will be essential for its realization.

In Conclusion

Restrictive measures in the fight against COVID-19 were very clearly relaxed throughout 2022 in Cambodia and in most Southeast Asian countries, which has enabled a very clear recovery of all IPC activities. A return to normality took place during the second quarter of 2022. Field activities could resume, as well as our scientist's various missions. We were also able to welcome visitors normally again.

Within the scope of our perspectives for the next two years, some important projects are set to be either finalized or launched:

- The development of bioinformatics through the recruitment of qualified personnel and the training of young Cambodian scientists;
- The establishment of a grant office to lighten the workload of scientists in setting up and monitoring projects;
- The consolidation of the IPC's quality assurance policy, by ensuring that the accreditations of 3 previously accredited entities are maintained, and by initiating the process for a number of other research units;
- A rearrangement of the MBL's opening hours for the public, with longer and/or daily time slots;
- The continuation of our PhD support policy, for the training of young nationals;
- The organisation of our Scientific Council meeting as soon as possible, following the turnover of key scientific personnel;
- For human resources, the completion of our staff's evaluation process, and the completion of the salary policy;
- The strengthening of the Preventive Medicine Service, made necessary by the increase in staff numbers;
- The strengthening of computer security in order to minimise hacking risks;
- The reflection on the renovation of the campus (project 2030) must be deepened and its result presented to the Ministry of Health and to the Institut Pasteur in Paris.

Finally, 2023 will see 70 years of presence of our Institute in Cambodia materialize, and we will organize events to celebrate this anniversary of the Institut Pasteur du Cambodge.

IPC is making continuous progress, and I would like to thank all personel for their efforts and excellent work, which allows our Institute to accomplish its mission for a better public health. I also wish to express my respectful gratitude to all national and international partners and membres of the Liaison Concil for their invaluable support, which helps us greatly in our work.

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

3. Institut Pasteur du Cambodge in 2022

3.1 General Presentation 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 signed in 1992, and since modified through two amendments. 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 also falls under the purview of Institut Pasteur in Paris, France, as regards its scientific and technical approaches and plans.

The Institute is part of the Pasteur Network (PN), a worldwide network of 33 members united by Pasteurian values, which contributes to the improvement of global health.

Governance

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 MoH of the Kingdom of Cambodia. The Deputy Director of IPC is named by the Director, in consultation with the MoH from within the pool of Cambodian scientists serving in the national public service, and who have doctorate degrees in the biological field or in public health.
On an annual basis, the Institute's activities are reviewed by the Liaison Council, presided by His Excellency the Minister of Health of Cambodia. The Council is composed of ten high-ranking members of the Cambodian Government or from its universities. The Director General of the Institut Pasteur in Paris, the Ambassador of France to Cambodia, and representatives of key international organizations in the health sector (WHO, UNICEF) complete the Council's membership.
Scientific activities are also reviewed every two or three years by the Scientific Advisory Board, its last session having been held in early February 2021. The scientific strategy is then revised, based on the recommendations from both the Liaison Council and the Scientific Advisory Board.
In addition, at the begining of each year, the Chief Financial Officer (CFO) sends the previous year's consolidated financial statements to the *Institut Pasteur* (International Division). An external financial audit is also performed in April of each year.

3.2 Organisation

The organisational chart is shown 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 Research (MR), Epidemiology and Public Health (EPH), Immunology (IMMUNO), Medical and Veterinary Entomology (ENTOMO), and Virology (VIRO);

- Health services, including the Medical Biology Laboratory (MBL), a Laboratory of Environment and Food Safety (LEFS), and a Vaccination Service (VACC) which includes three anti-rabies treatment centers and an International Vaccination Center;
- Public health laboratories, comprising 3 WHO Reference Centres hosted in the Virology Unit (the National Influenza Center in Cambodia, the WHO's Regional H5 Reference Laboratory, and the WHO's COVID-19 Global Referral Laboratory);
- A Voluntary Confidential Counselling and Testing service for HIV (VCCT), and rabies centers at three different sites that provide pre- and post-exposure prophylaxis at a fee.
- Four technical platforms: 1) an MSL-3 Laboratory, 2) a biobank, 3) a sequencing platform, and 4) flow cytometry.

3.3 Human Resources

As of 31 December 2022, the Institute had a team of 293 personnel:

- 273 with IPC contracts (of which 56 are MoH civil servants);
- 9 Cambodian scientists (2 Research Directors and 7 Research Fellows);
- 29 expatriates, including 10 with institutional contracts (one MEAE, seven IPP, one IRD, one from the French Agricultural Research and International Cooperation Organization-(CIRAD); and 19 on IPC contracts, including three PhD students).

IPC has personnel of 14 different nationalities, with 90 % being Cambodian nationals.

Diversity and Leadership

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

3.4 Finances and investments

3.4.1 Finances

The majority of IPC's revenue comes from specific research contracts funded by donors, from payments for services offered by IPC, including those preventing and treating rabies, and from a subsidy from the *Ministère de l'Enseignement Supérieur, de la Recherche, et de l'Innovation* (French Ministry for Higher Education, Research and Innovation, MESRI) via the Institut Pasteur in Paris. The Royal Government of Cambodia does not directly fund IPC, but it provides a significant contribution in the form of a tax and customs exemption.

Details on the different revenue streams are shown in table 1 and the figures 1 and 2 below represent the distribution of funding by country and by donor in 2021.

The share of income attributable to services remains high in 2022, as a result of funds earned by providing COVID-19 tests (especilally for travelers, COVID-19-recovery RT-PCR tests for hospitals and other public health facilities). The other income mainly originates from foreign exchange gains generated by the strong fluctuation of the Euro/USD exchange rate in 2022.

Table 1. Revenue Breakdown by Source (2012–2022)

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

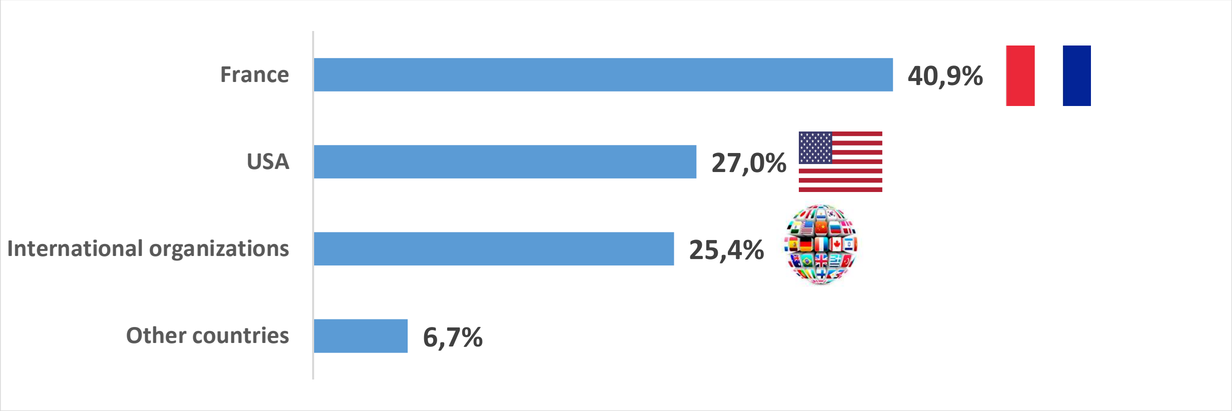


Figure 1. Breakdown of the reseach funds received per country

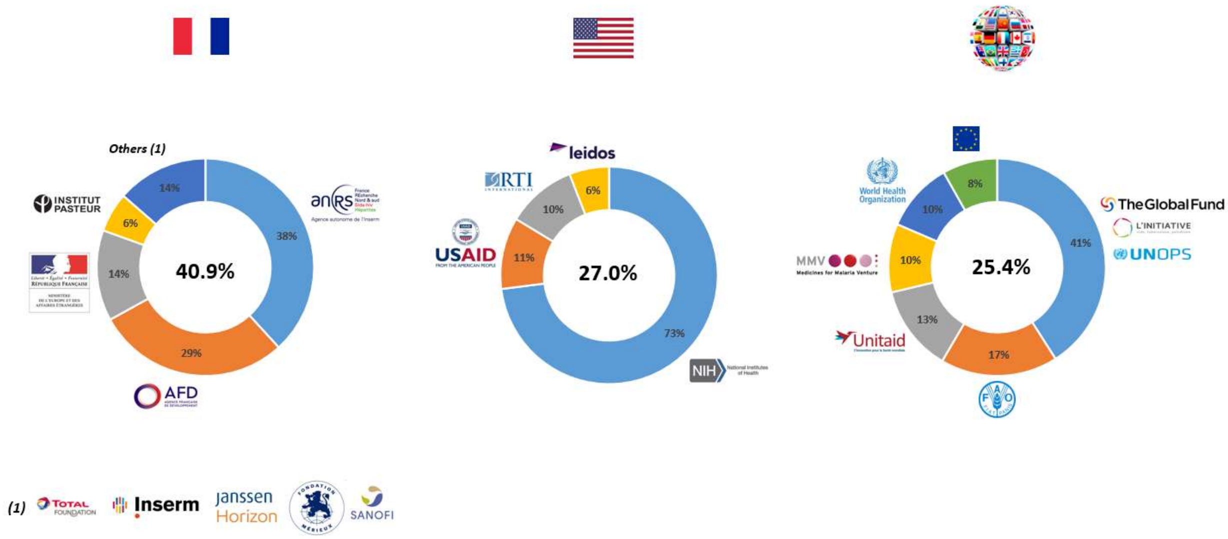


Figure 2. Revenue Breakdown by Source (2012-2021)

3.4.2 Investments into our Equipment and Facilities

Investments in Laboratory Equipment

Investments in laboratory equipment represented more than €850,000 in 2022
GIZ has provided more than €350,000 in laboratory equipment for the Virology Unit, particularly cell imaging instruments, fume hoods and -80ºC freezers.
The Immunology Unit has acquired a Chromium Controller for cell analysis, worth nearly €60,000 (NIH funding).

Finally, the MBL has purchased a Kingfisher (nucleic acid purifier) for nearly €45,000.

Reorganization of the Flow of Entries into the IPC

A new entrance was put into service in January 2022 (figure 3), thus meeting a triple objective:

- Secure access by distinguishing and separating the public area (MBL access, Rabies Prevention Center) from the area devoted to IPC research activities (secure access, lighting, video control, etc.).
- Improve the quality of reception with the recruitment of a team of receptionists positioned at the Campus' entrance, thus making it possible to better manage the flow of visitors.
- Create a building dedicated to COVID sampling for travelers and isolated from the flow of other visitors.

This work was financed partly with support from the United Kingdom (30 %) and partly with our own funds (70 %).

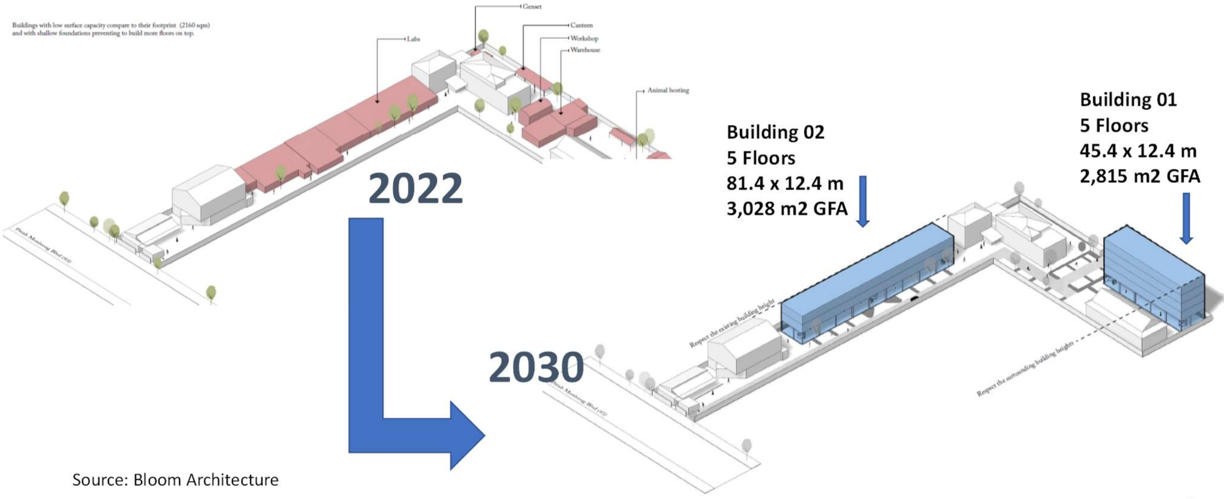
Figure 3. View of the IPC's entrance (Bloom Architecture)

Study on a Renovation of the IPC Campus: "IPC-2030 Project"

The Institute remains very attractive, and its numbers are growing every year. One of the factors limiting the IPC's development is the narrowness of its technical premises and of the campus in general. An evaluation was undertaken in 2022 by the Bloom Architecture firm, which carried out the modification of the IPC's accessways. The firm's conclusions confirmed that the buildings original foundations and the evolution of urban planning do not allow the laboratories to be raised by either one or two floors. In a long-term vision, it will therefore be necessary to completely rebuild one or more of our buildings.

This study has highlighted the site's potential, in particular through the construction of two new buildings, an increase in parking spaces, the opening of an access route at the rear of the site, new circulations pathways, more green spaces, and buildings favoring sustainable development (figure 4). This project will have to be developed in phases in order to preserve business continuity.

More specific studies will be continued in 2023 in order to determine the needs of our operational staff, and to develop a timetable and a first draft. This ambitious "IPC 2030" project will be presented to the Ministry of Health, whose opinions, authorizations and support will be essential for its realization.



Source: Bloom Architecture

Figure 4. View of the new Campus (Bloom Architecture)

3.5 Publications in 2022

IPC's research and public health activities are detailed in following sections. A summary of these is presented in figures 5 and 6 below. Research activities carried out in 2022 comprise the publication of 53 articles by scientists affiliated to IPC, and appearances in peer-reviewed international journals with impact factors (IF) higher than 0. Among these appearances, 19 were as first or last author, and 34 had an IF greater than or equal to 4.

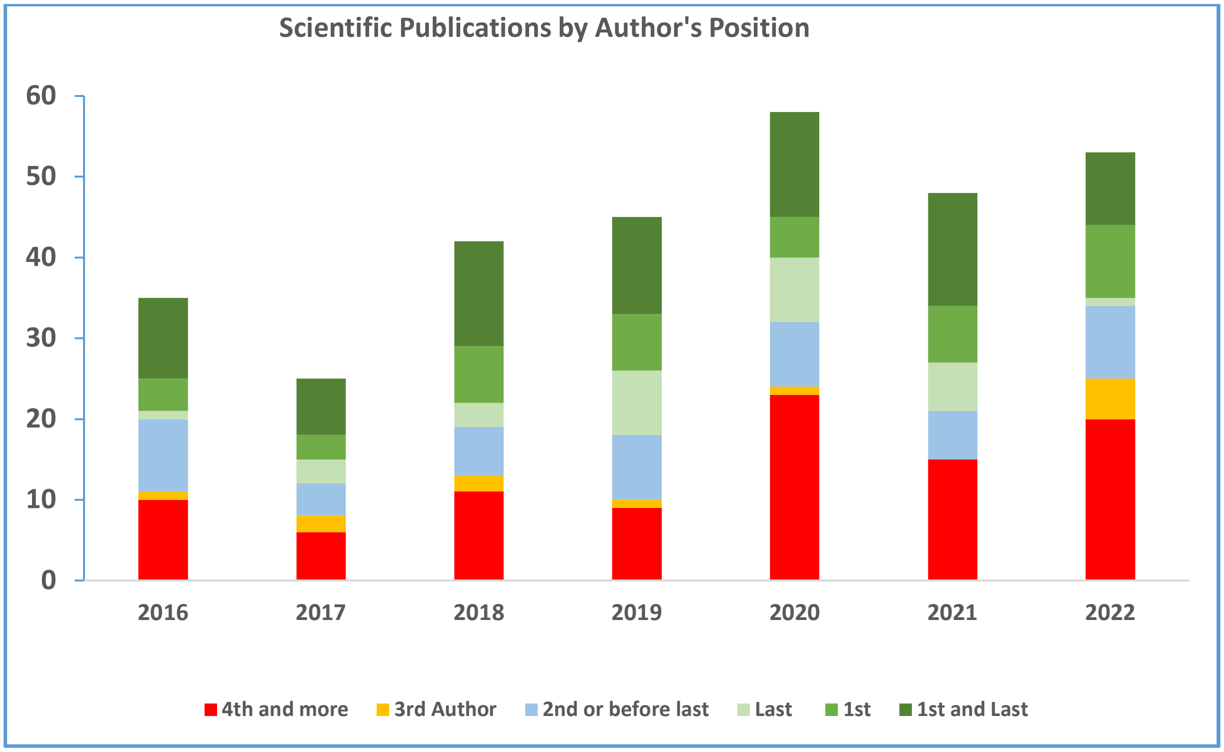


Figure 5. Scientific Publications by Author’s Position (2016–2022)

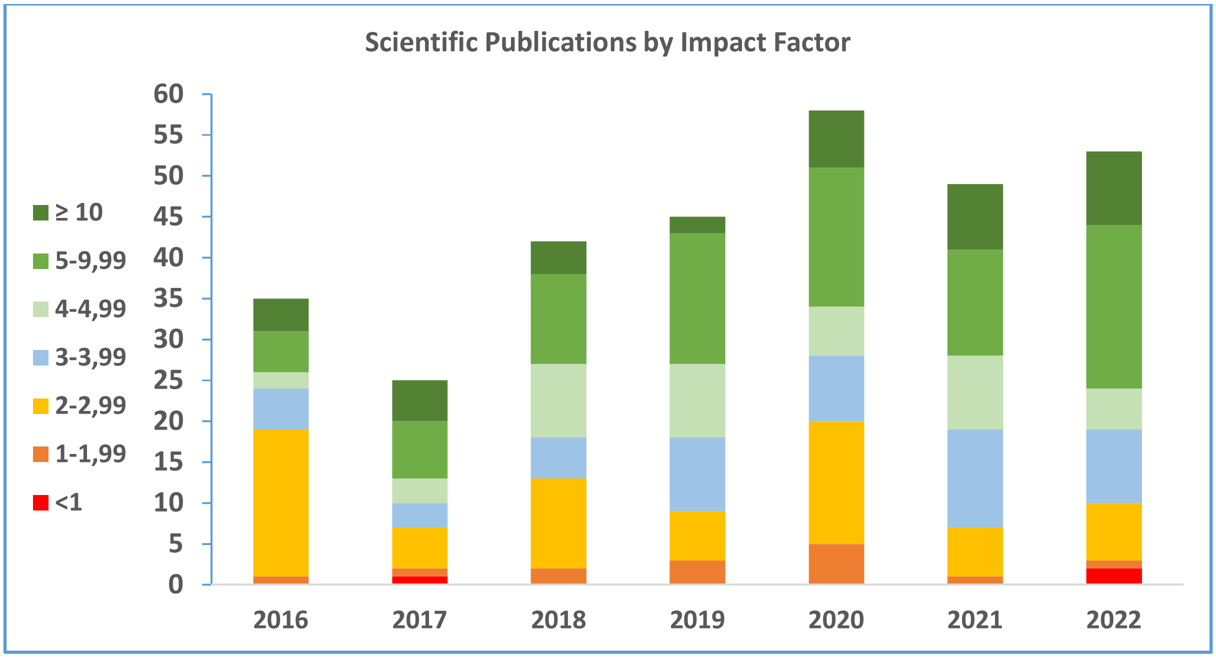


Figure 6. Scientific Publications by Impact Factor of the Journal (2016–2022)

3.6 Training and Internships 2022

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 Sciences (UHS) in Phnom Penh, and welcomes many students for internships and practical experiences.

Student Internships

In 2022, 73 students interned at IPC. This is higher than the 55 hosted in 2021, which number was limited due to the continuing COVID-19 epidemic. Among the 2022 students, 59 were Cambodian nationals, while the others were either French, Indonesian, Thai or Columbian. Their university affiliations are as follows: University of Health Sciences (23), University of Puthisastra (12), Institute of Technology of Cambodia (9), Royal University of Phnom Penh (7), while the remainder (26) were from various foreign universities in France, Belgium, and Germany. Eleven were PhD students (all Cambodian), 15 were in master's level studies, 31 were bachelor-level students, and 6 were working towards associate's degrees.

International Master's in Infectious Diseases

In 2022 as further in the past, IPC provided important and substantive support to the International Master's Programme in Infectious Diseases ("infectiologie" in French), a two-year programme jointly offered by UHS and *l'Université Paris Saclay* (UPS). Throughout the year, IPC was involved in the building of curriculum content (lectures, practical teaching, internship supervision), and by granting IPC scholarships to enrolled Cambodian students (2 scholarships are awarded per programme year). Due to travel restrictions, all teaching units were organized entirely remotely for the 2020–2021 school year. In-person teaching has resumed in 2022 and, since then, IPC also accommodated students in its premises for a moderate fee. IPC scientists contribute more than 200 hours of training per year.

The 2022–2023 International Master's Programme welcomed 14 students of 7 nationalities (including 4 Cambodians) for the M1 programme, as well as 9 students of 6 nationalities (including 1 Cambodian) for the M2 programme. As of 2023, since it was launched in 2019, a total of 47 students were admitted to this International Master's Programme.

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

	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

Other than providing financial support to the UHS, IPC has appointed 1 Senior Scientist and 1 Project Manager as focal points for the Master's Programme to facilitate communication between UHS and UPS. Namely, these are Dr Jean Popovici, Head of Research in the Malaria Molecular Epidemiology Unit, and Emilie Carlot, Project Manager.

Scientific Seminars

IPC has held scientific seminars every two weeks. Nineteen were held in 2022 (see Table 3).

Table 3: List of the 19 seminars held in 2022

Jan-5	Jurre Siegers	Post-doc researcher	Virology	Human and avian influenza A viruses in Cambodia during the SARS-CoV-2 pandemic, 2020-2021
Jan-26	Heang Vireak	Research engineer	Sequencing plateform	Next Generation Sequencing, Illumina Miseq Platform
Feb-09	Helene Guis	Researcher	EPHU / CIRAD	Dynamics and distribution of arboviral vectors in the Indian Ocean
Feb-23	Antsa Rakotonirina	Post-doc researcher	MVEU	The use of MALDI-TOF MS in medical entomology
March-09	Juliette Hayer	Post-doc researcher	LBM - Guest	impact of Insect Specific Viruses (ISVs) on vector competence in mosquitoes using omics approaches
March-16	Sotherary Sann	PhD Student	Immunology Unit	Regulatory T cells in dengue patients
March-23	Dim Bunnet	Researcher	EPHU - Clinical Research Group	Immunoglobulin-free strategy to prevent HBV mother-to-child transmission in Cambodia: the ANRS 12345 TA-PROHM study
April-06	Sudipta Hyder	Master Student	Virology Unit	Impact of COVID-19 on poultry farming and AIV risk in Cambodia
April-27	Jean Popovici	Researcher	Malaria Research Unit	Longitudinal analysis of PvDBP gene amplification and its impact on Plasmoidum vivax immune evasion in a Cambodian cohort
June-03	Laurent Renia	Prof	Singapore Immunology Network - Guest	Immunobiology of SARS-CoV-2 natural infection and vaccination: lessons from Singapore.
June-08	Eliott Benichou	Master 1 Student	Immunology Unit	A set of exosomal miRNA as biomarkers of IRIS in HIV/TB co-infected patients
June-06	Mahé Liabeuf	Master 2 student	EPHU / CIRAD	Evaluation of the wildlife surveillance system coordinated by WCS
July-09	Claude Flamand	Head of Unit	EPHU	Studying spatiotemporal dynamics of pathogen transmission using age-stratified and multiplexed serological survey data

Set-09	Marine Viglieta	PhD Student	EMVU /Guest	Impact of arboviruses co-infection on viral transmission by mosquitoes
Oct-12	Julia Guillebaud	Research assistant	Virology Unit	An overview of the ZooCoV project
Oct-19	Doeurk Bros	Master 2 student	EMVU	Ecological characteristics and temporal dynamics of mosquito species in Mondulkiri province
Nov-02	Emanuele Gioacchino	Post-doc researcher	Immunology Unit	Adaptive immune responses in clinical dengue
Nov-17	Gary Wong	Post-doc researcher	Virology Unit Guest	The importance of research into neglected, obscure, unknown pathogens and its impact on outbreak preparedness
Dec-14	Jurre Siegers	Post-doc researcher	Virology Unit	Environmental sampling and viral metagenomics : a tool to expand and enhance genomic surveillance at high risk interfaces.

3.7 Visits and missions at IPC in 2022

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

Table 4. Visits, missions and delegation received at IPC in 2022

Jan-20	Ms. Minh-Di TANG, Deputy Director for the Southeast Asia, Asia and Oceania Departments, French Ministry for Europe and Foreign Affairs
Jan-21	M. Pierre SOETARD, *France Volontaires*, Regional Head (Asia – Indian Icean – Pacific)
March-17	Ms. Clarisse VEYLON-HERVET, Regional Counsellor in global health Southeast Asia, French Embassy in Thailand
March-28	Ms. Catherine QUILLET, Project Manager-DATURA
March-31	Ms. Maria LOSADA, *Université Paris-Cité*
April-1	Prof. SAPHONN Vonthanak, Rector of the University oh Health Sciences, Phnom Penh.
April-7	Visit of the Association « Phnom Penh Accueil »
April-22	HE M. Jacques PELLET, French Ambassador in Cambodia
May-3	M. François ROGER, CIRAD's Regional Director for Southeast Asia
May-12	Mme Laurence SALUER, *L'initiative-Expertise France*
May-13	Delegation from *Deutsche Gesellschaft für Internationale Zusammenarbeit* (GIZ). held by Corinna HEINEKE (head of the Social Protection and Health programme).
May-18	Delegation from SingHealthDuke-NUS Global Health Institute, Singapore held by Prof. TAN Hiang Khoon, Director of SingHealth Duke-NUS Global Health Institute
May-22/25	Prof. Stewart COLE, CEO Institut Pasteur (Liaison Council)
June-6	Ms. Sarah BONNET, Institut Pasteur Paris

June-8	M. Laurent TOULOUSE, Deputy Director of Higher Education and Research, Department of Culture, Education, Research and Network, French Ministry for Europe and Foreign Affairs
June-15	Ceremony of signature of an agreement between UNIDO (United Nations for Industrial Development Organization) and IPC, Mr SOK Narin, Country Representative at UNIDO, Dr SHETTY Seetharama Thombathu, Chief Technical Adviser, HE POUM Sotha, Delegate of the Royal Government of Cambodia, Director General of the Fisheries Administration
Sept-20	Mr Jean-Pierre MARCELLI, Regional Director of AFD (French Agency for Development) for the East Asia Region.
Sep-22	Ms Cora ROOS, Ms Ulrike LECHNER and Ms Katharina TELFSER. Delegation from the *Physikalisch-Technische Bundesanstalt*, Germany
Oct-03	Dr Didier LAUREILLARD, DATURA project, France.
Oct-07	Dr Gary KOBINGER, Director, Galveston National Laboratory, Texas, USA.
Oct-21	Profs. Robert HECHT and Albert KO, Yale Institute for Global Health
Oct-21	Delegation from the International University (Phnom Penh) led by HE M. Barom, Vice-President
Nov-16	Delegation from the French National Research Institute for Sustainable Development (IRD) led by Mrs Valérie VERDIER, Chairwoman of the Board and CEO.

3.8 Institutional Issues, Objectives and Outlook (2023–2027)

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

3.8.1 Maintain the IPC's Position in its National and Regional Scientific and Medical Context

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

3.8.2 Develop its Partnerships with Scientific Institutions

Develop Partnerships with National Institutions

Partnerships with Cambodian Ministries (Agriculture, Education, Environment, and others beyond the MoH) and their affiliated institutions, including but not limited to, research centers, hospitals, universities, and the private sector, are important to the IPC in achieving its goals and objectives. Reinforcing and expanding these partnerships and collaborations would be to the advantage of IPC. In light of the similarity and complementary of the IPC and the UHS's work scopes, even closer collaboration could be considered in the fields of epidemiology and clinical research, particularly as regards the work done by teams associated on ANRS site in Cambodia.

Maintain and Strengthen the Hosting of Scientists from Outside Institutions, most Notably from CIRAD and IRD

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

3.8.3 Guiding Scientific Strategy

Organize the Eighth SAB Meeting

The last Scientific Advisory Board (SAB) meeting was held virtually in January 2021. To respect the defined 24-36 interval, and taking into account changes having happened at IPC, it would be beneficial to organize the next SAB during the last quarter of 2023, or during the first half of 2024.

Develop a 5-Year Strategic Plan

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

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

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

Develop and Expand Capacity in Bioinformatics

A working group was created in April 2021, regrouping various related skills existing at IPC. With the partnership between Institut Pasteur and the Doherty Institute (University of Melbourne, Australia), we have been able to extend of Mr. Koen VANDELANNOOTE's (a bioinformatician) contract for two years, and the "Bacterial Phylogenomics Group" was created in the beginning of 2023, for a period of two years.

Discussions evidenced the need to recruit a senior scientist specialized in this area. The strategy would be to work towards having bioinformatics specialists in each of the Institute's research areas. A senior bioinformatician will be recruited in 2023.

3.8.4 Reduce the Amount of Administrative Tasks Required of Scientists

The development of the IPC has been accompanied in recent years by an increased number of scientists and of grants requested and obtained from demanding donors (NIH, Wellcome Trust, Bill & Melinda Gates Foundation, etc.) for specific projects. This endeavour of monitoring calls for projects, of submitting, implementing, and coordinating projects administratively and financially requires more and more of our scientists' time, often to the detriment of their research. Hence, it appears necessary to support them in these tasks by creating a new structure adapted to this mission at the IPC, namely the "Grant Office" (GO).

This lightening of the administrative burden is necessary to provide more research time to scientists. Serious consideration should be given to the creation of a "Grant office" that would assist scientists in the development and follow-up of projects and proposals. IPC has sought advice from the Institut Pasteur de Dakar, whose size approximates IPC's, and which has implemented such a structure. This objective is in line with recommendations 4 and 5 from the last SAB, and we will attempt to create our Grant Office in 2023.

It is also foreseen that a new procurement system (automated) will be put in place that will integrate purchasing, billing and accounting. This would make the procurement process simple, decentralized, faster and more efficient.

3.8.5 Continue the Development of Quality Standards

IPC currently has three accredited units (MBL, LFSE and the Metrology Laboratory). The Virology Unit, due to its size and the critical activities it carries out (diagnosis of public health importance), must initiate a quality assurance program in 2023–2024. Given the difficulties of recruiting competent people in the field of quality assurance, the IPC must develop a strategy aimed at recruiting technicians, engineers, and pharmacists, and organizing a training course for them, enabling them to occupy the functions of quality manager within 12 to 18 months in accredited entities or on the way to accreditation.

3.8.6 Capacity Building for the IPC's Staff, and Making it a More Attractive Place to Work

Strengthening the IPC's Scientists' General Capacity

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

Strengthening Management Skills

We aim to continue ongoing efforts to build professionalism and skills in management, including supervision, mentorship.

Promoting the IPC's Staff Career Progression, and Making Careers More Attractive

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

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

Improving Workplace Safety and Security

The Consultative Committee on Safety and Security needs to be relaunched, and Preventive medecine for IPC's staff needs to be better structured.

Improving the IPC Infrastructure: IPC 2030 Project

The growth and evolution of activities over the last few years has led to an increase in the workforce, and the IPC's infrastructure that will soon reach its limits. It is time to seriously consider the need for new specialized space in the next five to ten years. In line with these new needs, a study of the "IPC 2030" project was carried out (cf point 2.3.2).

3.8.7 Fine-Tune the IPC's Financial Model

IPC is a not-for-profit organization. Its financial model rests on the successful pursuit of research and institutional grants, and on earning revenue through the offering of 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 is measured in "B" units, according to the French Nomenclature for Biological Acts, which makes it possible to quantify the activity. This activity remains at a level that is too modest, given the excellence of its technical platform and its potential to allow even more attractive prices.

IPC's laboratory is facing increasing competition from non-accredited laboratories, and also suffers from accessibility constraints due to the lack of parking space for patients. The establishment of an off-site sample-taking center 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 manager in January 2023. An extension of opening hours should take place in 2023.

70th Anniversary of the IPC (1953–2023)

In 2023, events to celebrate the 70th anniversary should be organized to celebrate the IPC's 70-year presence in Cambodia, in the service of science and health.

4. 2022 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, following the departure of Benoit Witkowski, who directed the Unit (then called Malaria Molecular Epidemiology Unit) between 2017 and 2022. The Unit encompasses four thematic areas: clinical studies, molecular biology & epidemiology, liver-stage biology and blood-stage biology.

The Unit is composed (as of January 2023) of one Head of Unit (J. Popovici–IP permanent researcher), one Deputy Head (Khim Nimol–IPC permanent researcher), three postdoc researchers (Costanza Tacoli, Brice Feufack and Agnes Orban), one medical supervisor (Eng Virak), one PhD student (Seng Dynang), and fourteen 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 scheme.

Malaria Research Unit
Head: Dr. Jean Popovici
Deputy Head: Dr. Nimol Khim
Secretary: Oudom Meas

Researchers & students	Clinical studies & field work	Molecular biology & epidemiology	Liver stage biology	Blood stage biology
Post-docs: Dr. Costanza Tacoli Dr. Brice Feufack Dr. Agnes Orban **PhD:** Dynang Seng **Master:** Rotha Eam Sivkeng Ouk Lea Baldor	Dr. Virak Eng Sitha Sin Sopheany Thin	Sophy Chy Chanvong Kul Malen Ea	Eakpor Piv Chantrea Vong Chansophea Chhin Sreyvouch Phen	Nimol Kloeung Sopheakvatey Ke

Figure 7. Malaria Research Unit Organigram

4.1.2 Research Programs – Major Achievements in 2022

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. The main achievement was the description of parasite resistance profiles collected as part of therapeutic efficacy studies in Cambodia, Vietnam, Laos and Papua New Guinea (PNG). Several hundreds of samples were characterized in order to provide an accurate epidemiological picture of the resistance. The parasites from Cambodia showed good susceptibility profiles to current first-line treatment and, despite an increase of *P. falciparum* cases in some parts of the country in 2022, we did not observe any sign of selection of multi-resistant parasites. A decrease in piperaquine-resistant parasites was described in Vietnam indicating that the change of national treatment guidelines is showing its efficacy in controlling these multi-resistant parasites. In Laos, we documented a recent increase in artemisinin-resistant profiles as well as ongoing selection of mutant genotypes in drug resistant genes, suggesting ongoing selection of multi-resistant parasites. Similarly, in PNG, we documented the emergence of artemisinin-resistant profiles, and future studies will be conducted to evaluate the response of parasites to these antimalarial drugs.

A secondary objective was to determine the population structure of the parasites circulating in the GMS. Preliminary results showed a remarkable drop in the parasite's diversity. In addition to its implications for surveillance, this research program builds on previous research activity on drug resistance in Cambodia. This aspect facilitates a better understanding of the mechanisms of counter-selection within the parasite population and led to a very important conclusion on the benefits of treatment policy set by Cambodian authorities in 2017. Data generated through this project is still being analyzed, but the main results have been shared with the WHO.

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

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, 8,000 molecules have been screened using our platform. This activity is financed by MMV. In parallel, we have developed a research program—financed by the National Institute of Health (NIH)—with our Ugandan partner to explore the most promising HITS family, better understanding their mechanism of action and evaluating their potency to become future effective and safe treatments.

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

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 103 patients have been enrolled so far. The study is still ongoing, and results will be presented in a future report.

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

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 completed the analysis for PvRBP2b and have demonstrated that none of the antibodies targeting this parasite protein allow for in-vitro neutralization. Similarly, while blocking the receptor of PvRBP2b on the surface of the immature erythrocyte (the transferrin receptor) allows the inhibition of PvRBP2b-RBC binding, we have shown that it does not prevent in-vitro invasion by parasites. Our data suggest that PvRBP2b is dispensable for parasite invasion and might not be worth pursuing as a vaccine candidate by itself. We have also shown that combining both targets (PvRBP2b and PvDBP) does not improve the parasite neutralization by anti-PvDBP. Our work on PvAMA1 and PvRBP2a was initiated recently and is ongoing.

Name	RBC Invasion Blocking
Funding	NIH
Project duration	2020-2025
External collaborator	Case Western Reserve University (CWRU) (Prof. Chris L. King), WEHI (Prof. James BEESON, Prof. Wai-Hong THAM)

Axis 3: Host-Parasite Interactions

Immune evasion of *P. vivax*

In 2020, we determined that *pvdbp* amplification was associated with an immune escape mechanism in parasites (Popovici & Roesch et al. Nat Com, 2020). This suggested a possible selection of amplified parasites by host anti-PvDBP immunity. We hypothesized that if this gene amplification is an immune evasion mechanism selected by host immunity, we should: 1) at the population level, observe a higher proportion of parasites carrying the gene amplification in areas where prevalence is higher (and therefore where more individuals would be immune), 2) at an individual level, observe a higher proportion of parasites with the *pvdbp* amplification collected in both immune asymptomatic individuals and symptomatic ones, and observe a higher proportion of amplified parasites in individuals with naturally acquired antibodies blocking the PvDBP-Duffy interaction, as well as individuals without such abs (referred to as BIabs). The analyses are almost complete and will be presented in a future report. We will also expand this data by analyzing parasites collected from a different geographical and epidemiological context (Ethiopia).

Name	Immune Evasion
Funding	NIH
Project duration	2020-2025
External collaborator	Case Western Reserve University (CWRU) (Prof. Chris L. KING), UNCC (Dr Eugenia LO), WEHI (Prof. Ivo MUELLER)

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

We know very little on 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 prepared late 2022, and the project will continue in the coming years.

Name	Pv Growth
Funding	NIH
Project duration	2022-2027
External collaborator	University of Maryland (Pr. David SERRE)

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 International Center of Excellence in Malaria Research (ICEMR, Program Director: I. Mueller/ L. Robinson), which aims to address the key challenges to malaria elimination in the Asia-Pacific. A coordinated set of in-depth studies into the epidemiology, entomology, and biology of residual malaria transmission are being carried out in three sites spanning the entire Asia-Pacific transmission gradient, from moderate and high transmission in Papua New Guinea to low, highly focal transmission in Cambodia. In order to provide a comprehensive picture of malaria epidemiology we have established a cohort of 650 individuals in Mondulkiri Province. The follow-up was completed in late 2020, and the results analyzed in 2021 were based on polymerase chain reaction (PCR). This data enabled us to identify several aspects of the malaria dynamic. The first point is the quasi-eradication of *P. falciparum*, consistent with the dramatic drop of documented cases. This suggests that the theory of a reservoir represented by asymptomatic carriers does not align with observed conditions. Conversely, we observed a persistence of *P. vivax* with an unchanged prevalence in asymptomatic infections, despite the number of symptomatic cases reducing by a factor of 5 over the last two years. Importantly, the indicators from our cohort showed that the populations experienced symptomatic infections as well, but that those were mainly undetected because of low parasitic densities. Altogether, this data suggests that *P. vivax* persists in a reservoir untargeted by current control measures.

To better quantify this reservoir, a serology-based measurement of the population's exposure to *P. vivax* is ongoing. We tested plasma samples for the presence of antibodies against a panel developed by Longley et al. (Nat Med 2020) which identifies recent exposure to *P. vivax*. All samples from the baseline (N=949) have been analyzed via the Luminex assay. The epidemiological analyses of these data are almost complete.

We have also analyzed 471 plasma samples collected at M12 and 565 samples from the additional collection at M21 of the longitudinal cohort. Analyzes of these data are ongoing.

To better understand the different vector species' ecology and their respective roles in malaria transmission, we established mosquito collection sites in forests and fields in a district of Mondulkiri Province, Northeastern Cambodia. In October 2021, 20 sites were established. A total of 3801 *Anopheles* females were collected. *Anopheles dirus* was the main collected species (n=2518), followed by *Anopheles hyrcanus* (n=273) and *Anopheles barbirostris* (n=129). From other primary vector species, 37 *Anopheles maculatus* and 5 *Anopheles minimus* were collected. A total of 1577 mosquitoes were collected in field sites, and 2224 in forest sites, with large differences in mosquito densities between the sites. Overall, 18.4 % of mosquitoes were collected during daytime, with less mosquitos collected during daytime in field sites (9 %) compared to forest sites (25 %; chi-square test P<0.0001). The forest canopy might be a more suitable environment for mosquito activity during daytime compared to the usually more opened field sites.

All samples were screened for *Plasmodium* infection, and 2.2% (n=84) of the mosquitoes were infected by *P. vivax* malaria parasites. Infected females were mainly collected in forest sites (57/84) especially in the site FOHO5, which was classified as a site with high human passage. *Anopheles dirus* was the main vector, representing 76 % of the infected mosquitoes. For the infected mosquitoes, 31 % were collected during daytime. Another mosquito collection was carried out in October 2022, and the mosquito species identification and molecular screening of malaria parasites is ongoing.

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

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 selected three villages to do this pilot study. Two interventions were performed 6 months apart (one in July 2022 and one in December 2022), and we performed cross-sectional surveys before and after the interventions. Three villages nearby were selected as controls without intervention. Analyses are ongoing.

Name	RAI3 SEROTAT
Funding	Global Fund
Project duration	2020-2023
External collaborator	WEHI (Pr. Ivo MUELLER), CNM (Dr. LEK Dysoley)

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 are developing a MinIon pipeline to perform this sequencing in malaria-endemic areas. Analyses are ongoing.

Name	G6PD seq
Funding	ACREME
Project duration	2022-2023
External collaborator	WEHI (Pr. Ivo MULLER), Menzies (Pr. Ric Price)

4.1.3 Research Programs – Outlook for 2023

Research Plan Overview

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

- Axis 1: Chemotherapy of Malaria Parasites
- Axis 2: Pre-Clinical Vaccine Development for Plasmodium Vivax
- Axis 3: Host-Parasite Interactions
- Axis 4: Operational Research for Malaria Elimination

Axis 1: Chemotherapy of Malaria Parasites

Drug Resistance Epidemiology……………………..………………..…...……………………….Funding: WHO

The issue of drug resistance in Asia has become less severe due to the decrease in cases, however we have demonstrated its emergence in the Asia-Pacific region and in Africa, and the matter remains highly relevant. The Drug Resistance Epidemiology program in GMS will remain, as the emergence of new strains could jeopardize malaria control measures, while investigations in other sites (Africa, Asia-Pacific, Central Asia) will increase, as the early emergence of drug resistance has been factually identified. Funding has been secured to perform these activities.

New Drug Candidates………………………………….……………………..…Funding: MMV/UGA, MMV
The antimalarial therapeutic approaches still present some important gaps that need to be filled. These are mainly focused on *P. vivax* anti-hypnozoite approaches and on *P. falciparum* resistance. *P. vivax* is still widely present in Asia, Africa (eastern and Madagascar), and South America, while ACT resistant *P. falciparum* is now emerging in Africa. Therefore, our efforts toward the identification of molecules suitable to these conditions will be maintained. The funding necessary to perform these activities is already in place.

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

Axis 2: Pre-Clinical Vaccine Development for Plasmodium Vivax

Blood-Stage Vaccine Candidates………………………………………………………….……Funding: NIH
Our capacity to perform short-term blood-stage cultures of P. vivax enables us to evaluate the invasion neutralization by antibodies targeting parasite antigens. One of our projects will therefore continue this rigorous evaluation in order to identify strain-transcending vaccine targets. Funding has been acquired to perform these activities.

Transmission-Blocking Vaccine. ……………………………………………………………..Funding: Oxford
Thanks to our capacity to perform experimental infections of mosquitoes, we are able to evaluate the ability of antibodies targeting sexual stage parasite antigens to block the transmission from humans to mosquitoes. Funding has been acquired to perform these activities.

Axis 3: Host-Parasite Interactions

Receptor-Ligand Interactions Involved in Duffy-Negative Red Blood Cell Invasion……Funding: NIH
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 University of North Carolina at Charlotte, 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. Funding has been secured to perform these activities.

Factors Involved in P. Vivax Growth………………………………………………………………Funding: NIH
We know very little on the factors influencing the growth of P. vivax in its human hosts. We have started a project that aims at deciphering the contribution of human factors (specifically G6PD deficiency and HbE hemoglobinopathy) and of parasite genotypes on the erythrocyte invasion and the development of P. vivax. Using a combination of scRNA-seq, genotyping and in vitro phenotype,

we aim to better understand how the parasite develops in its host. Funding is in place to perform these activities.

Mechanisms of Parasite Dormancy and Biology of Relapses. ………………………….Funding NIH
The mechanisms driving the biology of hypnozoites are completely unknown. Thanks to the work of a PhD student (started in Oct 2022), 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. Funding is in place to perform these activities.

Axis 4: Operational Research for Malaria Eradication

ICEMR Program………………………………………………………………………………Funding source: NIH
A longitudinal cohort was established, and cross-sectional surveys were conducted in Mondulkiri, Eastern Cambodia. Additional cross-sectional surveys will be performed in the same area. We will investigate, in collaboration with IP and the Walter and Elizabeth Hall Institute (WEHI), different biological metrics that may enable a better understanding of epidemiology. A serological analysis will be performed to define the magnitude of malaria exposure in the population. Entomological investigations will continue to evaluate the distribution and behaviour of malaria vectors in the study area. All of this lab-based data will be associated with epidemiological information. We will also investigate parasite population structure through an amplicon sequencing approach. All this information should bring new insights on malaria epidemiology and will enable a better tailoring of specific control measures.

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

Improving Blood-Stage Diagnosis of P. Vivax………………………………………………Funding: MRU
One of the reasons for P. vivax's resistance to control efforts is the very poor performance of current diagnostic tools used in endemic areas to diagnose infections. We have shown that current RDTs have a sensitivity of 30-40% compared to PCR to identify P. vivax parasites. Thanks to the work of a new postdoc (started November 2022), we are exploring alternatives to improve these diagnostic tools. MRU funding is in place to perform these activities.

Developing a MinIon Approach for G6PD Sequencing……………………………Funding: ACREME
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 are developing a MinIon pipeline to perform this sequencing in malaria endemic areas. Funding is in place to perform these activities.

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 support to drug efficacy studies that are conducted yearly in Cambodia.

4.1.5 Teaching and Training

PhD students
Anais Pepey: Ecology of malaria transmission (defence in June 2022)
Seng Dynang: Study of the biology of hypnozoites and relapses in Plasmodium vivax (started in October 2022).

Master students
Baura Tat: Determination of the presence of Duffy protein among Duffy-negative individuals infected by Plasmodium vivax

Maisha Tanzim: Optimisation of PCR for MinION sequencing of G6PD gene in the context of P. vivax anti-hypnozoite therapy

Emma Jousseaume: Optimisation of in-vitro drug testing to assess the susceptibility to mefloquine of Plasmodium vivax in isolates from Western Cambodia and for the analysis of pvmdr1 polymorphism

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

Jean Popovici coordinated the TU Parasitology-Mycology of the Joint Master's offered by the UHS and the *Université de Paris-Saclay*. He provided a 1.5-hour lecture on parasitology first year master's student, a 4.5-hour tutorial for the same students on article analysis, and a 6-hour tutorial on parasitology-mycology during the year. He also provided 1.5 hours on antigenic variation in Plasmodium parasites for second-year master's students.

4.1.6 Publications List in 2022

NOTE

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

1. **Asia-Pacific International Center of Excellence in Malaria Research: Maximizing Impact on Malaria Control Policy and Public Health in Cambodia and Papua New Guinea**
   Leanne J. Robinson, Moses Laman, Leo Makita, Dysoley Lek, Annie Dori, Rachael Farquhar, Amelie Vantaux, Benoit Witkowski, Stephan Karl, Ivo Mueller.
   Am J Trop Med Hyg. 2022;107(4_Suppl):124-30. DOI: 10.4269/ajtmh.21-1324

2. **Efficacy of three anti-malarial regimens for uncomplicated Plasmodium falciparum malaria in Cambodia, 2009-2011: a randomized controlled trial and brief review**
   Dysoley Lek, Agus Rachmat, Dustin Harrison, Geoffrey Chin, Suwanna Chaoratanakawee, David Saunders, Didier Menard, William O. Rogers.
   Malar J. 2022;21(1):259. DOI: 10.1186/s12936-022-04279-3

3. **Evaluation of HRP2 and pLDH-based rapid diagnostic tests for malaria and prevalence of pfhrp2/3 deletions in Aweil, South Sudan**
   Emily Lynch, Tomas O. Jensen, Bachir Assao, Menard Chihana, Thadeous Turuho, Dan Nyehangane, John B. Manyok, Harriet Pasquale, Nimol Khim, Benoit Witkowski, Matthew E. Coldiron. Malar J. 2022;21(1):261. DOI: 10.1186/s12936-022-04280-w

4. **Metabolic, Pharmacokinetic, and Activity Profile of the Liver Stage Antimalarial (RC-12)**
   Yuxiang Dong, Yogesh Sonawane, Steven P. Maher, Anne-Marie Zeeman, Victor Chaumeau, Amélie Vantaux, Caitlin A. Cooper, Francis C. K. Chiu, Eileen Ryan, Jenna McLaren, Gong Chen, Sergio Wittlin, Benoît Witkowski, François Nosten, Kamaraj Sriraghavan, Dennis E. Kyle, Clemens H. M. Kocken, Susan A. Charman, Jonathan L. Vennerstrom.
   ACS Omega. 2022;7(14):12401-11. DOI: 10.1021/acsomega.2c01099

5. **Preclinical characterization and target validation of the antimalarial pantothenamide MMV693183**
   Laura E. de Vries, Patrick A. M. Jansen, Catalina Barcelo, Justin Munro, Julie M. J. Verhoef, Charisse Flerida A. Pasaje, Kelly Rubiano, Josefine Striepen, Nada Abla, Luuk Berning, Judith M. Bolscher, Claudia Demarta-Gatsi, Rob W. M. Henderson, Tonnie Huijs, Karin M. J. Koolen, Patrick K. Tumwebaze, Tomas Yeo, Anna C. C. Aguiar, Iñigo Angulo-Barturen, Alisje Churchyard, Jake Baum, Benigno Crespo Fernández, Aline Fuchs, Francisco-Javier Gamo, Rafael V. C. Guido, María Belén Jiménez-Diaz, Dhelio B. Pereira, Rosemary Rochford, Camille Roesch, Laura M. Sanz, Graham Trevitt, Benoit Witkowski, Sergio Wittlin, Roland A. Cooper, Philip J. Rosenthal, Robert W. Sauerwein, Joost Schalkwijk, Pedro H. H. Hermkens, Roger V. Bonnert, Brice Campo, David A. Fidock, Manuel Llinás, Jacquin C. Niles, Taco W. A. Kooij, Koen J. Dechering.
   Nat Commun. 2022;13(1):2158. DOI: 10.1038/s41467-022-29688-5

6. **Single-cell RNA sequencing of Plasmodium vivax sporozoites reveals stage- and species-specific transcriptomic signatures**
   Anthony A. Ruberto, Caitlin Bourke, Amélie Vantaux, Steven P. Maher, Aaron Jex, Benoit Witkowski, Georges Snounou, Ivo Mueller.
   PLoS Negl Trop Dis. 2022;16(8):e0010633. DOI: 10.1371/journal.pntd.0010633

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. The year 2022 was marked by the arrival of a new Head of Unit, Dr Claude Flamand, and a new Head of the Clinical Research Group, Dr Nathalie De Rekeneire, following the departure of Dr Laurence Borand. The functional structure of the Unit has also evolved, following the integration of the IRD's GeoHealth group into the Unit, the creation of a Methodology and Statistical Data Analysis Group, and the dissolution of the Malaria Group. The Unit is now composed of five research groups (Figure 7):

- **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 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 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 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 National Pediatric Hospital and the Kantha Bopha Children’s Hospital.

Robust partnerships with the University of Health Sciences and the Institute of Technology of Cambodia (ITC) were also strengthened through collaborative projects.

Finally, most research projects result from partnerships with international agencies or research groups including the *Agence nationale de recherche sur le SIDA et les maladies infectieuses émergentes* (ANRS-MIE), the Dengue Vaccine Initiative , the International Vaccine Initiative (IVI), the European Union, *Fondation Total*, *Institut national de la santé et de la recherche médicale* (INSERM), *L'Agence inter-établissements de recherche pour le développement international (AIRD)*, the International Division of Pasteur Institutes, *Institut Pasteur* in Paris, CIRAD, Pasteur Foundation, *MSD Avenir*, Gillings Public Health Fellowship, the World Health Organization, UNITAID, *L'Initiative (Expertise France)*, and the *Agence française de développement* (AFD).

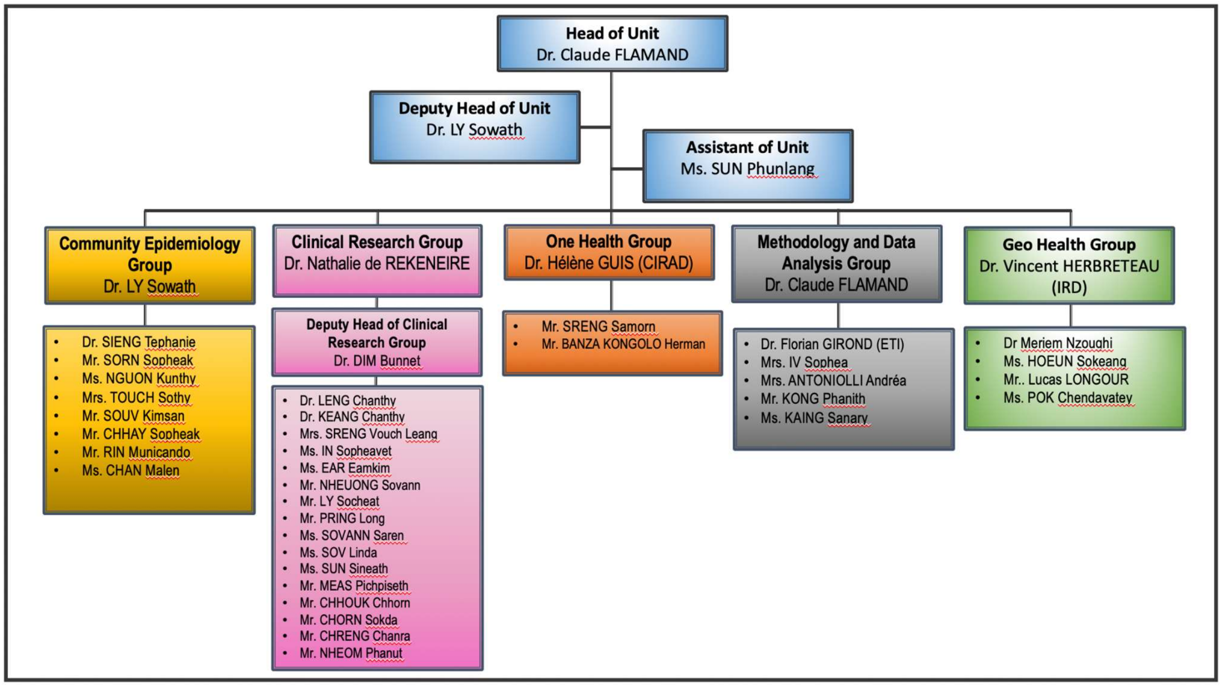


Figure 8. Epidemiology and Public Health unit organogram, 2022

4.2.2 Research Programs - Major Achievements In 2022

Axis 1: Viral Diseases

Rabies

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

This study aims to evaluate the protective antibody response's survival time, through the monitoring of a cohort of approximately 170 patients at day 14, at 6 months, at 1 year, and at 3 years after the first dose of the vaccine schedule, using the vaccination protocol described above.

Collaborations	Team Leader: LY Sowath. Partners: Immunology Unit (T. CANTAERT), Virology Unit (DUONG V.), Vaccination Unit (PENG Y.)
Funding	Institut Pasteur du Cambodge: (2019-2024)

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

This project describes the humoral immune response (rabies virus-neutralizing antibodies, or RVNA), measuring it with Fluorescent Antibody Virus Neutralization tests (FAVN) at different times in two study groups: at baseline before the first PEP dose, then at 14 days and 28 days after the first PEP injection.

Collaborations	Team Leader: LY Sowath. Partners: Immunology Unit (T. CANTAERT), Virology Unit (DUONG V.), Vaccination Unit (PENG Y.)
Funding	Sanofi Pasteur RAB00056 (2020-2022)

Immunity Persistence after Abridged Intradermal Rabies PEP: The RESIST-3 Study

The aim of this study is to assess whether the intradermal regimen (3 doses / 1 week) confers long-term immunity equivalent to that conferred by TRC intradermal regimen (4 sessions / 1 month), and to explore the characteristics of humoral and cell-mediated protection and response, before and after boosting.

Collaborations	Team leader: LY Sowath. Partners: Immunology Unit (T. CANTAERT), Virology Unit (DUONG V.), Vaccination Unit (PENG Y.), Institut Pasteur (P. PARIZE)
Funding	Action concertée inter-Pasteurienne (ACIP) n° 403-2020 (2021-2022)

Rabies – One Health

An extensive door-to-door longitudinal survey in Battambang and Kandal Provinces was carried out to estimate the demographics of dog populations, identify dog ownership determinants, and analyse dog management practices. The estimated yearly cumulative bite incidences, 3.1% and 2.3% in Battambang and Kandal respectively, were found to rank among the highest in the world. The dog population turnover was particularly high, with implications on dog vaccination strategies.

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

Arboviruses

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

Data on the clinical characteristics and consequences of CHIKV infections on obstetrical outcomes is scarce in countries with limited resources. The objective of this study is to characterise the clinical signs of CHIKV during pregnancy and obstetrical outcomes among confirmed CHIKV-infected pregnant women in one large hospital in Cambodia. Analysis of the results is ongoing.

Collaborations	Team Leaders: Laurence BORAND (Epidemiology Unit), Tineke CANTAERT and Alvino MAESTRU-NETTO (Immunology Unit), DUONG Veasna (Virology Unit), Olivier SÉGÉRAL, Jayavarman VII Hospital
Funding	ANRS-MIE - IPC

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. Laboratory follow-up of suspected cases will be performed.

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

DogZooSea
The DogZooSea project aims to improve knowledge and management of two major public health problems in Cambodia and neighbouring 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 1) documents dog owners' perceptions and management practices, 2) assesses dog distribution and exposure to flaviviruses in dogs and their owners, and 3) assesses the existence of non-lethal exposures to rabies in dogs.

Collaborations	Team Leader: LY Sowath Partners: Helene GUIS, SORN Sopheak (Epidemiology Unit), Heidi AUERSWALD (Virology Unit), Michel DE GARINE WICHATITSKY (Cirad-KU), Universitas Gadjah Mada (Yogyakarta, Indonesia), Faculty of Veterinary Technology of the Kasetsart University (Bangkok, Thailand), AUF
Funding	FSPI-One Health in Practice in Southeast Asia (OH SEA)

Coronaviruses

ECOMORE 2 Top-Up COVID
The Top-Up COVID project is a complementary component of the Ecomore 2 project, which provided a significant support to Ecomore 2-partner institutes, as well as Pasteur Network institutes in Vietnam, which were on the front line in the fight against COVID-19. This support provided by the Epidemiology Unit and the GeoHealth Group helped the institutes to implement surveillance activities at national or regional scales, and to implement their epidemic responses.

Collaborations	IPC-Virology (DUONG Veasna, Narjis BOUKLI), IPC-Epidemiology (LY Sowath, SORN Sopheak), IRD–GeoHealth (Vincent HERBRETEAU, Florian GIROND, Lucas LONGOUR)
Funding	AFD, 2020-2022 (complementary to ECOMORE 2)

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. Recruitment and follow-up of patients is ongoing.

Collaborations	Team leaders: 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)

TB-Speed Research Project

TB-Speed is a multi-centre (seven countries) research project, aiming at improving the diagnosis of childhood tuberculosis through decentralization of TB diagnosis and systematic tuberculosis diagnosis in vulnerable children. The study ended in July 2022, and a meeting for the restitution of the results with the national authorities was done in July 2022.

Collaborations	Team Leader: Laurence BORAND (Clinical Research Group) Partners: CENAT, National Pediatric Hospital, Kampong Cham and Takeo Hospitals, Batheay and Ang Rokar Districts' health facilities
Funding	UNITAID & L'Initiative/Expertise France (2019-2021)

OPTICAM: Optimizing Latent Tuberculosis Treatment Initiation in Cambodia among People Living with HIV

The aim of the project is to improve latent tuberculosis infection (LTBI) treatment uptake in people living with HIV (PLHIV), by assessing the impact of an alternative treatment intervention, as compared to the current practice of 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. Analysis of the results is planned during the first trimester of 2023.

Hepatitis

TA-PROHM - ANRS 12345: Tenofovir as Preventive Treatment for Hepatitis B Transmission in Mothers

This project aims to prevent mother-to-child transmission (MTCT) by reducing the HBV viral load in mothers by offering antivirals. The antiviral course typically begins at week 24 of the pregnancy. Patient recruitment and follow-up phases are completed. A meeting for the presentation of the results is planned in May 2023.

Collaborations	Team Leaders: Laurence BORAND (Clinical Research Group), Patrice PIOLA (Epidemiology Unit), DUONG Veasna (Virology Unit) Partners: Calmette Hospital, NMCH, Jayavarman VII Hospital, National
Funding	ANRS-MIE (2017-2021)

Bacteriological Diseases and Antibiotic Resistance

Water and Health Risks in Cambodia (Wat-Health)

Wat-Health aims at studying the exposure and vulnerability of rural populations to the most notable health hazards related to floods (leptospirosis, melioidosis, mosquito-borne diseases, exposure to pesticides), by conducting investigations in the Mekong Delta. In 2021, the GeoHealth group worked to describe the land use and land cover with very high-resolution satellite images. In 2022, it conducted a serological and KAP survey with the LBM to assess the prevalence of leptospirosis and melioidosis in the local population (500 individuals) and explore the risk factors associated with these two diseases. In 2023, GeoHealth is building the project's online platform, which will integrate and share information from its various components (bacteriology, entomology, hydrology).

Collaborations	Wat-Health coordination: Sylvain MASSUEL (IRD - U*MR G-EAU*); serological and KAP survey: Vincent HERBRETEAU (IRD – Epidemiology Unit) and LY Sowath (Epidemiology Unit), Mallorie HIDÉ (IRD - MIVEGEC, LBM DRISA), Gauthier DELVALLEZ (LBM); entomological survey: Sébastien BOYER (IPC); Hydrology: ITC and IRD
Funding	Fonds de soutien pour les projets innovants (FSPI), 2021-2022

A Hospital-Based Case-Control Study to Identify Risk Factors of Leptospirosis and to Improve Post-Disaster Management of Emerging Diseases (ECOMORE 2 - WP Myanmar)

This study helped to develop the diagnosis of leptospirosis in hospitals within the Yangon Metropolitan Area, in Myanmar. A hospital-based case-control study allowed to explore the socio-demographic and environmental risk factors of leptospirosis. The collaboration stopped due to a difficult political situation, but data analysis resumed in 2021. In 2021, the GeoHealth group developed an online application for the prediction of conditions suitable for the emergence of leptospirosis.

Collaborations	Team leader: Patrice Piola (Epidemiology Unit), Partners: NHL Myanmar (HTAY HTAY Tin, KHIN Nyein Zan, MAY July), Institut Pasteur de Nouvelle Calédonie (Cyrille GOARANT), IRD-Espace-Dev (Vincent HERBRETEAU, Sylvaine JÉGO, Lucas LONGOUR)
Funding	ECOMORE 2 (AFD 2017-2022) – WP Myanmar

FSPI ARCAHE

This activity is led by the LBM and LMI DRISA. The project's objective is twofold: 1) to identify the sources of emergence and spread of resistant bacteria in Cambodia using a "One Health" approach, and 2) to evaluate whether the MinION technology could be used as a diagnostic tool. The Epidemiology Unit was involved in the recruitment of hospitalized patients with bacterial infections (both resistant and non-resistant), while the Animal Work Package (Battambang) sampled animals in households from patients with antibiotic resistance.

Collaborations	Team leader: Véronique CHEVALIER (CIRAD), Partners: Patrice PIOLA (Epidemiology Unit). IRD (Anne-Laure BANULS, Mallory HIDE), Medical Biology Laboratory (CHENG Sokleaph, Gauthier DELVALLEZ), CIRAD (Véronique CHEVALIER), Calmette Hospital (BORY Sotharith), Battambang Hospital (CHIEK Sivhour)
Funding	Fonds de soutien pour les projets innovants (FSPI) – 2020-2022

Parasitological Diseases

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

The main reservoirs of parasites in Cambodia are inside its forests. This study, done in collaboration with MMEU, aims at an in-depth understanding of malaria transmission inside three forests totalling 200km² (year 1: 2019-2020) followed by an intervention (year 2: 2020-2021) to eliminate in-forest malaria. Malaria cases registered in neighbouring health centres were monitored to estimate the intervention's effectiveness.

Collaborations	Team leader: Patrice PIOLA Partners: EAV Sophea (Epidemiology Unit). Partners for Development (PfD), National Center for Parasitology, Entomology and Malaria Control (CNM), World Health Organization (WHO), Malaria Molecular Epidemiology Unit
Funding	L'Initiative Canal 2: 17SANIN205 – 2019-2021

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

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

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

4.2.3 Research Programs - Outlook for 2023

BCOMING (Biodiversity Conservation to Mitigate the Risks of Emerging Infectious Diseases) is a 6-million-euro project coordinated by CIRAD, which aims to better understand the relation between biodiversity loss in biodiversity hotspots and disease emergence, in order to prevent and react to future pandemics. It will be implemented in three tropical biodiversity hotspots with different environmental and socio-cultural settings in Southeast Asia (Cambodia), West Africa (Ivory Coast and Guinea) and the Caribbean (Guadeloupe). In Cambodia, the project will help to understand and prevent the emergence of SARS-CoV-2-related coronaviruses that have recently been detected in cave-roosting rhinolophid bats in the northern part of the country.

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

AFRICAM is a project developed in the framework of the Preventing Zoonotic Disease Emergence (PREZODE) initiative, coordinated by CIRAD and IRD in four African countries and in Cambodia. The main objectives of the project will be 1) to study the zoonotic risk at different interfaces between 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 BAÑULS (IRD - MIVEGEC), CoPi: Flavie GOUTARD (Cirad - ASTRE), Country focal point: LY Sowath (IPC), Cirad country focal point: H. GUIS, IRD country focal point: V. HERBRETEAU, Serological survey: C. FLAMAND in collaboration with IRD, CIRAD, IPC, Agronomes et vétérinaires sans frontières (AVSF), International Development Enterprises (IDE), ITC, Wildlife Conservation Society (WCS), Battambang Hospital.
Funding	AFD, 2022-2025

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

Collaborations	Scientific coordinators: Alexandre HOBEIKA (CIRAD), Adèle KACOU N'DOUBA (University of Abidjan) Country coordinator: LY Sowath (IPC)
Funding	French Ministry of Foreign Affairs, French Embassy (FSPI OH)

OHARAT (One Health Anthropological approach to RAT-related knowledge and practices in Cambodia and beyond in Southeast Asia)

By analysing the interconnection between people, animals, plants, and their shared environment, this one-year project aims to document how sociocultural local knowledge is linked with scientific knowledge. Ethnographic and multi-field research allowed researchers to investigate the social representation of rats (rural–urban perceptions, differences between sites and between species), zoonoses and health practices. The project held a two-day workshop at IPC (25 and 26 October 2022), with the participation of researchers from various fields related to rats (anthropology, ecology, virology, history, agriculture, geography).

Collaborations	Meriem M'ZOUGHI (IRD), Vincent HERBRETEAU (IRD)
Funding	FSPI One Health in South-East Asia (OHSEA, Fonds de soutien pour les projets innovants), 05/2022-03/2023

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. This study is currently under preparation.

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)

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 has just been funded and is under preparation.

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 (2023-2027)

4.2.4 Support to National Authorities

The following summarizes key support to Cambodian national authorities over the year 2022.

- 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/TB.
- Dim B. is involved with the CENAT in the preparation of the TB prevalence survey that will start during the first semester of 2023.
- L. Borand participated in the Elimination of Mother-to-Child Transmission of HIV, Syphilis and Hepatitis B preparatory workshops organized by the National Maternal and Child Health Center (NMCHC). She also took part in the Consultative Workshop on the Implementation of the National Strategic Plan and Financing for the Viral Hepatitis Program.
- Ly S. worked with the Emergency Operational Center (EOC) for Covid-19, coordinated by the Cambodian CDC-MoH.
- Ly S. and V. Herbreteau's teams provided support for data management for Covid-19 lab testing at IPC and ensured the transfer of quality data to the CDC-MoH.

4.2.5 Teaching and Training

Teaching, Training and symposium

- Lectures (Ly Sowath) for foundation year students at the University of Health Science
- Epidemiology and Clinical Trial Courses, (Claude Flamand): Master in Infectiology, *Université Paris-Saclay*, University of Health Sciences, 18 hours (M1 and M2)
- Training on "Mapping and Spatial Analyses in R for One-Health Studies", from 28 Nov to 2 Dec 2022, Frangipani Hotel, Phnom Penh, organized by Vincent Herbreteau (IRD-IPC) and Florian Girond (ETI) with Lucas Longour (IRD), Sokeang Hoeun (IPC), Timothée Giraud (CNRS) and Léa Douchet (IRD); 23 participants from 16 institutions and 6 Southeast Asian countries.
- Symposium on geospatial approaches in One-Health studies on 5 Dec, Himawari Hotel, Phnom Penh,
  organized by Vincent Herbreteau (IRD-IPC), Florian Girond (ETI), Lucas Longour (IRD), Sokeang Hoeun (IPC), Sylvie Laleu (IRD), Aung Myint Thu (SMRU) and Kevin Jung Yuan-Lee (SMRU); 59 participants from 8 countries, including 6 Southeast Asian countries. A website was created to organize the symposium and share information https://geoonehealth2022.sciencesconf.org

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)

Master's Students

- Dr Dim Bunnet (LSHTM Clinical Trials, 2019–2024).
- Mr Nheuong Sovann (UHS-Epidemiology, 2020–2023).
- Ms Sov Linda (Master's of Science in Epidemiology at NIPH, Cambodia, 2020–2023).
- Ms Yang Jee Seong (Master's in Public health, Pasteur-CNAM, 2021-2022).
- Ms In Sopheavet (Master's in Epidemiology, National Institute of Public Health, 2022-2024)
- Ms Sun Sineath (Master's of Health and Community Development, National Institute of Public Health, 2022–2024)
- Mr Long Pring (Master's in Epidemiology, National Institute of Public Health, 2022–2024)
- Ms Chan Malen (Master's of Applied Epidemiology - MAE, ASEAN–Australia Health Security Fellowship Program, National University of Australia - ANU, 2022–2023)
- Ms Mahé Liabeuf (Master's in Eco-Epidemiology, Montpellier University, 2022)

Student Internships

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

4.2.6 Publications List in 2022

NOTE

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

1. **Genomic epidemiology of SARS-CoV-2 in Cambodia, January 2020 to February 2021** Yvonne C F Su, Jordan Z J Ma, Tey Putita Ou, Leakhena Pum, Sidonn Krang et al.
   Virus Evol, 2022 Dec 16;9(1):veac121.

2. **Accessibility to rabies centres and human rabies post-exposure prophylaxis rates in Cambodia: A Bayesian spatio-temporal analysis to identify optimal locations for future centres.**
   Jerome N. Baron, Véronique Chevalier, Sowath Ly, Veasna Duong, Philippe Dussart et Didier Fontenille, Yik Sing Peng, Beatriz Martínez-López.
   PLoS Negl Trop Dis. 2022;16(6):e0010494.

3. **Aerosol exposure of live bird market workers to viable influenza A/H5N1 and A/H9N2 viruses, Cambodia.**
   Paul F. Horwood, Srey Viseth Horm, Sokhoun Yann, Songha Tok, Malen Chan, Annika Suttie, Phalla Y, Sareth Rith, Jurre Y. Siegers, Sorn San, Holl Davun, Sothyra Tum, Sowath Ly, Arnaud Tarantola, Philippe Dussart, Erik A. Karlsson.
   Zoonoses and Public Health. 2022. 70(2):171-175.

4. **Household clustering supports a novel chemoprophylaxis trial design for a mosquito-borne viral disease.**
   Hugh R. Watson, Veasna Duong, Sowath Ly, Marie Mandron, André M. Siqueira, Guilherme S. Ribeiro.
   Int J Infect Dis. 2022;122:169-73.

5. **Impact of the switch from four to three intradermal rabies post-exposure prophylaxis sessions in patients bitten by dogs: A cost-consequence analysis from the patients' perspective.**
   Alicia Le Bras, Kevin Zarca, Yiksing Peng, Malen Chan, Isabelle Durand-Zaleski.
   One Health 2022;15:100408.

6. **Influx of Backyard Farming with Limited Biosecurity Due to the COVID-19 Pandemic Carries an Increased Risk of Zoonotic Spillover in Cambodia.**
   Sudipta Hyder, Benjamin L. Sievers, Claude Flamand, Damian TagoPacheco, Malen Chan, Filip Claes, Erik A. Karlsson.
   Microbiol Spectr. 2022;e0420722.

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

8. **Temporal patterns of functional anti-dengue antibodies in dengue infected individuals with different disease outcome or infection history.**
   Hoa Thi My Vo, Vinit Upasani, Heidi Auerswald, Sokchea Lay, Sotheary Sann, Axelle Vanderlinden, Sreymom Ken, Sopheak Sorn, Sowath Ly, Veasna Duong, Philippe Dussart, Tineke Cantaert.
   Sci Rep. Nature Publishing Group; 2022;12(1):17863.

9. **Diagnostic Advances in Childhood Tuberculosis—Improving Specimen Collection and Yield of Microbiological Diagnosis for Intrathoracic Tuberculosis.**
   Eric Wobudeya, Maryline Bonnet, Elisabetta Ghimenton Walters, Pamela Nabeta, Rinn Song, Wilfred Murithi, Walter Mchembere, Bunnet Dim, Jean-Voisin Taguebue, Joanna Orne-Gliemann, Mark P. Nicol, Olivier Marcy.
   Pathogens. 2022;11(4):389.

10. **Effect of systematic tuberculosis detection on mortality in young children with severe pneumonia in countries with high incidence of tuberculosis: a stepped-wedge cluster-randomised trial.**
    Olivier Marcy, Eric Wobudeya, Hélène Font, Aurélia Vessière, Chishala Chabala, Celso Khosa, Jean-Voisin Taguebue, Raoul Moh, Juliet Mwanga-Amumpaire, Manon Lounnas, Veronica Mulenga, Sandra Mavale, Josina Chilundo, Dalila Rego, Bwendo Nduna, Perfect Shankalala, Uzima Chirwa, Agathe De Lauzanne, Bunnet Dim, Emeline Tiogouo Ngouana, Madeleine Folquet Amorrissani, Lassina Cisse, Flore Amon Tanoh Dick, Eric A. Komena, Sylvie Kwedi Nolna, Gerald Businge, Naome Natukunda, Saniata Cumbe, Prossy Mbekeka, Ang Kim, Chanrithea Kheang, Sokha Pol, Elizabeth Maleche-Obimbo, James A. Seddon, Tan Eang Mao, Stephen M. Graham, Christophe Delacourt, Laurence Borand, Maryline Bonnet, Olivier Marcy, Angeline Serre, Anne Badrichani, Manoa Razafimanantsoa, Julien Poublan, Aurélia Vessière, Clémentine Roucher, Estelle Occelli, Aurélie Beuscart, Aurélie Charpin, Gemma Habiyambere, Salomé Mesnier, Eric Balestre, Bandana Bhatta, Anne-Laure Maillard, Joanna Orne-Gliemann, Emmanuelle Baillet, Nicolas Koskas, Marc D'Elbée, Delphine Gabillard, Hélène Font, Minh Huyen, Maryline Bonnet, Manon Lounnas, Hélène Espérou, Sandrine Couffin-Cadiergues, Alexis Kuppers, Benjamin Hamze, Laurence Borand, Agathe

de Lauzanne, Bunnet Dim, Chanthy Keang, Long Pring, Song Yin, Channimol Sarith, Chanvirak Phan, Sovann Nheuong, Socheat Ly, Sanary Kaing, Vouchleang Sreng, Elen Lun, Leakhena Say, Sophea Suom, Romyka Ferhy, Dina So, Sorunna Born, Sophea Pal, Boraneath Nang, Tan Eang Mao, Ang Kim, Viso Srey, Piseth Kan, Leakhena Hout, Samnang Ith, Sophany Oum, Sokunvadhana Sau, Kim Heang Ho, Daronic Kith, Nathara Nuch, Chhun Leang Horm, Cheameas Sophon, Bosba Roeungdeth, Chhay Meng, Ravin Rith, Samnang Phy, Chanchetra Sor, Voleak Sao, Sophea Khat, Bunthoeun Mak, Angkeaborin Uy, Sreyny Khay, Kimsan Som, Rongvirak Hach, Hay Sok, Sotheavy Kuon, Synatt Heng, Amara Seng, Sopheak Nim, Reach Pan, Srean Kim, Keo SREY Leap, Bormey Net, Viccheka Noun, Daven Lay, Chhaing Many, Socheata Seng, Vuthy Ly, Saran So, Sovutthik Oun, Sopheap Chey, Rattany Chhea, Lydeth Baong, Vanna Thoung, Chanrithea Kheang, Borady By, Vathanak Nguon, Eksophea Meach, Sopheak Tek, Sina Ngeav, Tetra Lun, Deth Hem, Nayreang Chut, Setha Sarik, Hgekkoung Nang, Mengnean Meach, Sopal Sreng, Dara Sar, Rathana Kin, Phoran Ros, Chenda Dorn, Chansy Kak, Srey Leak Sambath, Leakhena Son, Linda Bin, Eangnay Pengong, Sokha Pol, Samnang Khutsorn, Sorsophea Seang, Virak Soun, Vuthy Vong, Chandara Khoeung, Panha Um, Sokunthea Bou, Sarin Song Pich, Puthy Nim, Sopheak Khat, Nuon Ban Si, Sovannodom Ream, Sim Ing, Phanith Chann, Samrith Ngeth, Marina Sun, Sokea Chhoeung, Soeun Sean, Ratanak Prak, Jean-Voisin Taguebue, Sylvie Kwedi Nolna, Audrey Amboua Schouame Onambele, Numfor Hycenth, Bernard Melingui, Angeline Nkembe Medounmga, Luciole Hougnang Tatmi, Nathalie Etemgoua, Vanessa Kouesso, Jean Bugin, Celestine Nzedjom, Roger Ngoya, Jules Eyike, Elyse Loudjom, Roger Lonsti, Ladi Dang, Edward Bintar, Chantal Njayong, Cinthia Ngonsoa O, Isabelle Ndzeukap, Pascaline Dzoyem, Clémentine Dzokou, Berthe Dindo, Raoul Moh, Eric Auguste Komena, Roger Aka Bony, Christian Kouadio, Serge Danho, Melissa Goli, Madeleine Folquet, Max Valère Itchy, Abdel Sidibé, Lancina Cissé, Joseph Ouattara, Mamadou Konaté, Flore Amon-Tanoh Dick, Melissa Cardena, Laurence Adonis-Koffi, Djabia Eugenie, Ferdinand Kouamé, Hervé Menan, André Inwoley, Timothée Ouassa, Marcelle Sandrine Nguessan, Celso Khosa, Saniata Cumbe, Emelva Manhiça, Alcina Zitha, Valter Chiúle, Eva Muxanga, Irene Gune, Yara Lima, Jorge Ribeiro, Sandra Mavale, Josina Chilundo, Felismina Maxanguana, Natália Morais, Julieta Manhiça, Josefina Give, Jafito Atumane, Gelson Lucas, Arsénio Thai, Adélio Chave, Dalila Rego, Lúcia Guambe, Faiaz Issa, Rosa Carneiro, Neusa Pene, Natércia Florindo, Dália Machel, Cecília Cumbane, Helena Mendes, Mule Kitungwa, Valdo Muianga, Humberto Tamele, Adelino Sulude, Roda Mabota, Herquéria Comandante, Abelardo Massangaie, Eric Wobudeya, Gerald Bright Businge, Faith Namulinda, Robert Sserunjogi, Rashidah Nassozi, Charlotte Barungi, Hellen Aanyu, Doreen Muwonge, Eva Kagoya, Serene Aciparu, Sophia Chemutai, Samuel Ntambi, Amir Wasswa, Juliet Nangozi, Abner Tagoola, Prossy Mbekeka, Sajja Kenneth, John Paul Lubega, Aidah Nassali, Jessica Tagobera, Christine Agwang, Florence Kalembe, Annet Ajambo, Elizabeth Aguti, Samuel Kasibante, Henry Matende, Israel Owen Odongo, Juliet Mwanga Amumpaire, Naome Natukunda, Gertrude Ngabirano, Paul Kakwenza, Simpson Nuwamanya, Miria Nyangoma, Jane Nabbuto, Florence Abok, Rinah Arinaitwe, Diana Birungi, Evans Mwesigwa, Daniel Atwine, Hassan Mbega, Patrick Orikiriza, Ivan Taremwa, Esther Turyashemererwa, Hope Derrick, Dan Nyehangane, Rodney Kaitano, Susan Logoose, Steven Businge, Charles Ntambi, Jerome Mugabi, John Mzee, Julius Besigye, Saul Kanzira, Phionah Turyatemba, Florence Twebaze, Chishala Chabala, Veronica Mulenga, Perfect Shankalala, Chimuka Hambulo, Vincent Kapotwe, Marjory Ngambi, Kunda Kasakwa, Uzima Chirwa, Chifunda Kapula, Susan Zulu, Grace Nawakwi, Teddy Siasulingana, Jessy Chilonga, Maria Chimbini, Mutinta Chilanga, Bwendo Nduna, Muleya Inambao, Mwate Mwambazi, Barbra Halende, Wyclef Mumba, Endreen Mankunshe, Maureen Silavwe, Moses Chakopo, Roy Moono.
The Lancet Infectious Diseases. Elsevier; 2022;0(0).

11. **Excess risk of subsequent infection in hospitalized children from a community cohort study in Cambodia and Madagascar.**
    Lison Rambliere, Elsa Kermorvant-Duchemin, Agathe de Lauzanne, Jean-Marc Collard, Perlinot Herindrainy, Muriel Vray, Benoit Garin, Andrianirina Zafitsara Zo, Fanjalalaina Rasoanaivo, Jacob Rakotoarimanana Feno Manitra, Tanjona Bodonirina Raheliarivao, Jean-Baptiste Niokhhor Diouf, Véronique Ngo, Siyin Lach, Pring Long, Laurence Borand, Touch Sok, Armiya Youssouf Abdou, Michael Padget, Yoann Madec, Didier Guillemot, Elisabeth Delarocque-Astagneau, Bich-Tram Huynh
    International Journal of Epidemiology. 2022;dyac048.

12. **Immunoglobulin-free strategy to prevent HBV mother-to-child transmission in Cambodia (TA-PROHM): a single-arm, multicentre, phase 4 trial.**
    Olivier Segeral, Bunnet Dim, Christine Durier, Sovann Nhoueng, Kearena Chhim, Saren Sovann, Sophal Yom, Chanlina Vong, Song Yin, Bandith Ros, Vutha Ky, Sothy Pech, Bunthoeun Nem, Kay Hout, Julia Guillebaud, Eamkim Ear, Layana Caroupaye-Caroupin, Claire Rekacewicz, Laura Fernandez, Denis Laurent, Chantana Yay, Rattana Kim, Laurence Meyer, Samsorphea Chhun, Laurence Borand for the ANRS-MIE TA PROHM Study Group Chanthy Keang, Ousa Khan, Boraneath Nang, Vouch Leang Sreng, Sopheavet In, Sineath Sun, Linda Sov, Bunrachana Nor, Brembrey Hing, Sokkim Seng, Sophea Soum, Leakhena Say, Sao Sarady Ay, Daneth Thol, Chhorn Chhouk, Patrice Piola, Janin Nouhin, Anne-Marie Roque Afonso, Jean Charles Duclos Vallee, Channa Sann, Leang Sim Kruy, Maud Lemoine, Laurent Mandelbrot, Stephane Blanche, Alpha Diallo, Christelle Paul, SAY Tiv, Polinn Sar, Lyvoin Nov, Darapoline Vann, Tha Chea, Bunrith Touch, Kongkea Neav, Ekvitou Kong, Ratha Chea, Chanksolina Ouk, Lyhour Meak, Rayounette Krouch, Naneth Chhan, Sody Seang, Veasna Nuon, Leang Meng, Sok Leakhena Tharith, Sovannara Hang, Vanrithy Som, Rithy Som, Phirak Seng, Malys Lim, Kimchhorn Srey, Sok Rothavy Uch, Pichthyda Hou, Satha Bo, Eanghor Ieang, Kimchhorng Korn, Chan Reatrey Noun, Sokhoeun Soy, Thou Khim, Vutha Sou, Sokha Pol, Samreth Nget, Marina Sun, Phearom Uon, Kim Teng Ya, Kimsreng Lean, Kim Ean Eang, Sophal Ung, Rauin Rith, Charya Mom, Chanthea Keang, Soklyda Sam, Sokneth Chuong, Chanmony Nam, Sophya Khuon, Sidet Cheang, Sopheak Lean, Arnaud Tarantola, Isabelle Fournier, Nicolas Rouveau, Maria-Camila Calvo cortez.
    The Lancet Infectious Diseases. 2022.

13. **Knowledge, attitudes and practices on childhood TB among healthcare workers.**
    B. Joshi, H. Font, E. Wobudeya, M. Nanfuka, A. Kobusingye, J. Mwanga-Amumpaire, N. Natukunda, S. Turyahabwe, L. Borand, T. E. Mao, B. Dim, R. Ferhi, R. Moh, J. Kouakou, R. Aka Bony, G. Breton, A. Mustapha, L. Matata, L. Foray, A. Detjen, S. Verkuijl, M. Sekadde, C. Khosa, V. Mbassa, J-V. Taguebue, S. Kwedi Nolna, M. Bonnet, O. Marcy, J. Orne-Gliemann
    Int J Tuberc Lung Dis. 2022;26(3):243-51.

14. **Prevalence and factors associated with faecal carriage of extended-spectrum β-lactamase-producing Enterobacterales among peripartum women in the community in Cambodia.**
    Agathe de Lauzanne, Navin Sreng, Elsa Foucaud, Touch Sok, Thida Chon, Chhaily Yem, Veasna Hak, Sothada Heng, Meng Soda, Malika Gouali, Maya Nadimpalli, Malin Inghammar, Mamitina Alain Noah Rabenandrasana, Jean Marc Collard, Muriel Vray, Simon Le Hello, Alexandra Kerleguer, Patrice Piola, Elisabeth Delarocque-Astagneau, Didier Guillemot, Bich Tram Huynh, Laurence Borand, **BIRDY study group**: Andrianirina Zafitsara Zo, Marguerite

Diatta, Jean Baptiste Diouf, Fatoumata Diene Sarr, Joseph Faye, Sophie Goyet, Perlinot Herindrainy, Elsa Kermorvant-Duchemin, Siyin Lach, Veronique Ngo, Michael Padget, Feno Manitra Jacob Rakotoarimanana, Bodonirina Tanjona Raheliarivao, Frédérique Randrianirina, Abdoulaye Seck, Arnaud Tarantola, Armya Abdou Youssouf.
Journal of Antimicrobial Chemotherapy. 2022;dkac224.

15. **Tuberculosis Diagnosis in HIV-Infected Children: Comparison of the 2012 and 2015 Clinical Case Definitions for Classification of Intrathoracic Tuberculosis Disease.** Olivier Marcy, Sophie Goyet, Laurence Borand, Philippe Msellati, Vibol Ung, Mathurin Tejiokem, Giang Do Chau, Francis Ateba-Ndongo, Abdoul Salam Ouedraogo, Bunnet Dim, Paul Perez, Julien Asselineau, Guislaine Carcelain, Stéphane Blanche, Christophe Delacourt, Sylvain Godreuil.
Journal of the Pediatric Infectious Diseases Society. 2022;11(3):108-14.

16. **Modeling Japanese encephalitis virus transmission dynamics and human exposure in a Cambodian rural multi-host system.**
Héléna Ladreyt, Véronique Chevalier, Benoit Durand.
PLoS Negl Trop Dis. 2022 Jul 11;16(7):e0010572.

17. **Vector Competence of Mediterranean Mosquitoes for Rift Valley Fever Virus: A Meta-Analysis.**
Alex Drouin, Véronique Chevalier, Benoit Durand, Thomas Balenghien.
Pathogens. 2022 Apr 23;11(5):503.

18. **Transmission dynamics of Q fever in French Guiana: A population-based cross-sectional study.**
Sarah Bailly, Nathanaël Hozé, Sylvie Bisser, Aurélien Zhu-Soubise, Camille Fritzell, Sandrine Fernandes-Pellerin, Adija Mbouangoro, Dominique Rousset, Félix Djossou, Simon Cauchemez, Claude Flamand.
The Lancet Regional Health - Americas 2022; 16: 100385.

Abstracts and Presentations

1. **Simplified criteria to assess long-term antiviral treatment indication in chronic HBV infected pregnant women in Cambodia.**
Yang JS, 2022 Mekong Hepatitis Symposium, Vientiane, Laos, December 8, 2022. Online oral presentation.

2. **Progress towards achieving the elimination of Hepatitis C in the Southeast Asia Region. De Rekeneire N**
Mekong Hepatitis Symposium, Vientiane, Laos, December 8, 2022. Online oral presentation.

3. **Field experience of nasopharyngeal aspirate and stool sample collection for the detection of tuberculosis in children in six low-income and high-tuberculosis-incidence countries.**
G. Breton, J.R. Mugisha, M. Nanfuka, E.N. Massama, R. Moh, J.-V. Taguebue, L. Borand, C.J.J. Khosa, J. Mwanga, M. Bonnet, O. Marcy, E. Wobudeya, TB-Speed decentralization study working group. Oral presentation at The Union, 2022.

4. **Impact of decentralizing childhood TB diagnosis approaches at the primary health centre and district hospital levels for case detection in high TB burden countries.**
   E. Wobudeya,M. Nanfuka, M. Huyen Ton Nu Nguyet, J. Mwanga-Amumpaire, J.-V. Taguebue, G. Breton, R. Moh, C. Khosa, L. Borand, J. Orne-Gliemann, M. Bonnet, O. Marcy, TB-Speed Decentralization Group. Poster presentation at The Union, 2022.

5. **Clinical characteristics and management of children below 5 years old admitted for very severe community-acquired pneumonia in Africa and Southeast Asia.**
   A. de Lauzanne, B. Dim, E. Mao Tan, C. Chabala, C. Khosa, R. Moh, J.-V. Taguebue, J. Mwanga-Amumpaire,M. Bonnet, E. Wobudeya, O. Marcy, L. Borand, TB-Speed Pneumonia Working Group. Poster presentation at The Union 2022

6. **Training peripheral health staff and monitoring quality of chest X-ray interpretation for TB diagnosis in children through quality assurance.**
   P.-Y. Norval, E. Leroy-Terquem, B. Melingui, S. Danho, J. Ribeiro, R. Arinaitwe, B. Flomo, S. Nhoeung, E. Balestre, M. Bonnet, O. Marcy, E. Wobudeya.
   Poster presentation at The Union 2022.

7. **Leptospirosis seen from the space: tracking suitable environments for leptospirosis transmission to inform early surveillance.**
   Herbreteau V, Longour L, Jégo S, July M, Goarant C, Piola P, Mouquet P, Révillion C, Bouche D, Girond F.
   12th Scientific Meeting of the International Leptospirosis Society (ILS), Thailand: Bangkok, 14/11/2022.

8. **Estimating leptospirosis burden in Southeast Asia and its future evolution based on climate and environmental determinants.**
   Douchet L, Goarant C, Mangeas M, Menkès C, Hinjoy S, Herbreteau V.
   12th Scientific Meeting of the International Leptospirosis Society (ILS), Thailand: Bangkok, 14/11/2022.

4.2.7 Outlook

The research strategy for the next few years aims to develop, according to the priorities identified in Cambodia, useful research programs to inform targeted public health strategies. Particular emphasis will be placed on the training and capacity building of teams in different areas (clinical research, data management, biostatistics, geomatics) through in-house and university training (Master's and PhD degrees) for the unit's staff. It will also be important to attract talented Cambodian researchers and international postdoctoral scientists and to extend regional collaborations in order to promote the implementation of ambitious and collaborative projects to support national health authorities through relevant research findings. Given the importance of public health issues in Cambodia, it will also be important to identify opportunities to extend research themes to non-communicable diseases (respiratory diseases, cardiovascular diseases, cancers, etc.), and to establish a specialized social science division within the unit, aimed at implementing socio-anthropological approaches in projects carried out within the unit.

4.3 Immunlogy 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 four major research axes: 1) the investigation of immunopathological mechanisms during arbovirus infection, 2) the identification of new immune-related biomarkers for infectious diseases, 3) the immunological assessment of rabies post-exposure prophylaxis, and 4) the investigation of adaptive immune responses in SARS-CoV-2 infected patients. Moreover, we are hosting a transversal single-cell analysis platform.

4.3.1 Organizational Structure

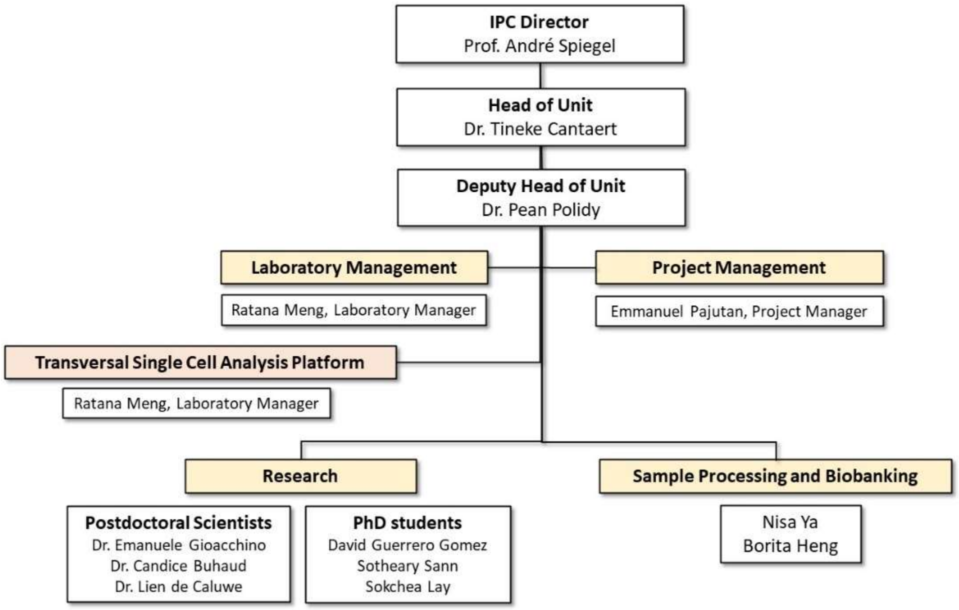


Figure 9. Immunology Unit Organigram

4.3.2 Research Programs – Major Achievements in 2022

Axis 1: Investigation of Immunopathological Mechanisms during Arbovirus Infection

Our current work on arbovirus infections is focused mainly on understanding the immunopathology of dengue. 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 in populated urbanized areas, contributing to the spread of dengue. Outcomes after infection with dengue vary greatly between individuals. Our previous work comparing the immune response in asymptomatic acute infected individuals with hospitalized patients revealed profound differences in the adaptive immune response profile associated with a different clinical outcome to infection (Simon-Loriere et al., Scie Transl Med, 2017, Bournazos et al., Science 2021).

Aim 1.1 Humoral Immune Responses to Dengue Virus

The humoral immune response during heterotypic secondary DENV infection is complex and dynamic, and consists of a polyclonal response of pre-existing antibodies against the previous infecting serotype and newly generated antibodies.

Therefore, we assessed the contribution of the developing humoral immune response in the course of acute infection in Cambodian children with either asymptomatic infection or dengue disease requiring hospitalization (Figure 10). Percentages of antibodies bound to DENV-infected cells are associated with disease outcomes and severity after infection. ADE-mediating antibodies evolved over time and were highest against the infecting serotype. As a whole, this data indicates that binding antibodies might contribute to protection or pathogenesis via other mechanisms not related to neutralization or enhancement of infection (Vo et al., Sci rep 2022).

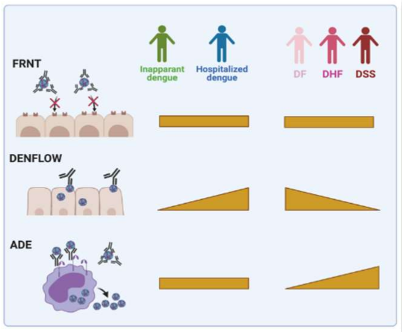


Figure 10. Detailed assessment of the humoral immune response during secondary DENV infection with 3 cell-based assays. The foci reduction neutralization assay (FRNT) measures neutralizing antibodies, DENFLOW measures antibodies bound to infected cells and the antibody-dependent enhancement assay (ADE) measures the balance of the antibodies' neutralizing and enhancing activities (Vo et al., Sci Rep 2022).

Start/End Year	2017-Ongoing
Collaborations	Virology Unit, IP du Cambodge (DUONG V), Epidemiology and Public Health Unit, IP du Cambodge (LY S)
Funding	HHMI/Wellcome International Research Scholars Program (208710/Z/17/Z) 2017-2022

Aim 1.2 Immunity to Aedes Mosquito Saliva

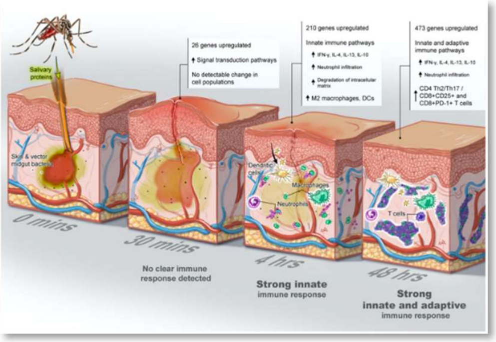


Figure 11. Graphical abstract of the changes in skin immune populations in humans after Aedes mosquito bites. (Guerrero, Vo et al, Nat Comm 2022)

When a mosquito inserts its proboscis and probes for blood, the mosquito ejects a salivary mix of vasodilators, anticoagulants, immunomodulatory and anti-hemostatic components into both the epidermis and the dermis (reviewed in Cantaert T and Manning J, Vaccines, 2019). However, little is known about skin immunity to mosquito saliva. We assessed the cutaneous innate and adaptive immune responses via controlled *Aedes aegypti* feedings in humans living in an Aedes-endemic country. Gene expression profiling and immunophenotyping revealed induction of neutrophil degranulation and recruitment of skin-resident dendritic cells and M2-macrophages. As the immune reaction progressed over time, T cell priming and regulatory pathways were upregulated along with a shift to a Th2-driven response and CD8+ T cell activation. Accordingly, the participants' bitten skin cells produced less pro-inflammatory cytokines when stimulated by *Aedes aegypti* salivary gland extract. These results identify key immune genes, cell types, and pathways in the human response to mosquito bites that can be leveraged to develop novel therapeutics and vector-targeted vaccine candidates to arboviral diseases. (Guerrero, Vo et al., Nat Comm 2022).

Start/End Year	2020-2023
Collaborations	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

Axis 2: Biomarkers of Infectious Diseases

Aim 2.1 Agence Nationale de Recherches sur le SIDA et les Hépatites Virales (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 patients (miRBio)"

MicroRNAs are reported as powerful regulators of post-translational gene expression and can act as biomarkers in several infectious diseases. Host miRNAs target certain HIV genes, affecting HIV replication thus thereby participating in viral control. In HIV elite controllers, a set of expressed miRNA can characterize this clinical phenotype. Several studies reported the characterization of miRNA expression profile in tuberculosis (TB) patients, but evaluation of miRNA expression in co-infections such as TB/HIV are lacking. In this study, we evaluate by flow cytometry whether a circulating miRNA pattern might be used as potential biomarkers in HIV/TB coinfection and to correlate the miRNA expression profile of 27 selected miRNAs with the clinical evolution and the occurrence of IRIS. We found that the combination of at least two or three microRNA markers (MiRBio0004, MiRBio0001 and MirBio0008) can be used as the biomarker to differentiate IRIS from non-IRIS before any treatment initiation and predict the occurrence of IRIS. 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, 2017-2019

Aim 2.2 ANRS no. 12394: "Lowering InterLeukin-1 receptor antagonist concentrations after TB treatment onset: a proof-of-concept study in Cambodia and Ivory Coast (LILAC-TB)"

Additional tools are urgently needed not only to help diagnose TB but also to assess the response to TB treatment in empirically treated patients. In a previous study, we found that Interleukin-1 receptor antagonist (IL-1Ra) plasma concentrations dropped dramatically after two months of TB treatment. The objective of this proof-of-concept study is to demonstrate that IL-1Ra concentrations significantly decrease 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 at week one after the treatment was similar. The manuscript is in preparation.

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, 2019-2021

Axis 3: Immunological Responses to Rabies Virus Vaccination

The World Health Organization endorsed a new shortened protocol of post-exposure prophylaxis (PEP) in its April 2018 guidelines. This *"Institut Pasteur du Cambodge* protocol" of three PEP sessions of two-site intradermal 0.1 ml vaccine doses each at days 0, 3, and 7 is the first one-week PEP regimen to be recommended (Cantaert T., Borand L. et al., Lancet Infect Dis 2019). The "IPC protocol" is to date the shortest and most vaccine-sparing rabies PEP protocol approved by the WHO. An evaluation of the protection and antibody responses of this newly WHO-endorsed vaccination protocol is necessary. Therefore, we aim to monitor safety, efficacy, and protection (as measured by anti-rabies virus-neutralizing antibody responses and rabies-specific T cell responses) in patients up to three years after the newly WHO-endorsed "IPC protocol" vaccination regimen.

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

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

The duration of humoral and cellular immune memory following SARS-CoV-2 infection in populations in least developed countries remains understudied but is key to overcoming the current SARS-CoV-2 pandemic. Sixty-four Cambodian individuals with laboratory-confirmed infection with asymptomatic or mild/moderate clinical presentation were evaluated for spike-binding and neutralizing antibodies and for antibody effector functions during the acute phase of infection, and at a 6 to 9-month follow-up. Antigen-specific B cells, CD4+ and CD8+ T cells were characterized, and T cells were interrogated for functionality at late convalescence. Anti-spike antibody titers decreased over time, but effector functions mediated by spike-specific antibodies remained stable. Spike-specific B cells could be detected in late convalescence in the activated memory B cell compartment and are mostly IgG+. CD4+ and CD8+ T cell immune memory was maintained to S and membrane (M) protein. Asymptomatic infection resulted in decreased antibody-dependent cytotoxicity and frequency of SARS-CoV-2-specific CD4+ T cells at late convalescence. Whereas anti-spike antibodies correlated with spike-specific B cells, there was no correlation between T cell response and humoral immune memory. Hence, all aspects of a protective immune response are maintained up to nine months after SARS-CoV-2 infection and in the absence of re-infection. (Vo, Maestri et al., Frontiers Immunol 2022).

Start/End Year	2020-2022
Collaborations	Virology Unit, IP du Cambodge (DUONG V), Epidemiology and Public Health Unit, IP du Cambodge, Virus and Immunity Unit, IP Paris (BRUEL T)
Funding	Institut Pasteur « COVID-19 urgence » - 2020-2021

4.3.3 Research Programs – Outlook 2023

Axis 1: Immune Responses during Arbovirus Infection

Aim 1.1 Unravel B-Cell Responses in Asymptomatic Dengue-Infected Individuals

In this project, we aim to understand the mechanism of the generation and protection of the humoral immune response in very mild dengue cases, compared to hospitalized dengue cases with classic dengue fever and hospitalized cases with severe dengue on a single-cell level. We will evaluate the development, quality and quantity of the humoral responses in detail. To accomplish this goal, we work on the following specific aims: 1) generation of dengue-derived antigens from clinical field isolates; 2) determination of antibody affinity for four key-dengue antigens in individual plasmablasts via droplet microfluidics to define on a single cell level the cross-reactivity and epitope specificity of antibody-secreting cells; and 3) single-cell RNA sequencing of CD19+ B cells in different dengue-infected patient groups. For this project, all experiments have been performed and we will focus on data analysis and manuscript writing for the year 2023.

Start/End Year	2019-2022
Collaborations	Virology Unit, IP Cambodia (DUONG V), Structural Virology Unit, IP Paris (BARBA-SPAETH G, FLAMAND M) Antibodies in Therapy and Pathology Unit, IP Paris (BRUHNS P), CB UTECHS, IP Paris (HASAN M)
Funding	PTR (PTR 2019-2022), Janssen Horizon grant: 2019-2022

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

Other than virus neutralization, many other functions are attributed to antigen-specific antibodies in the 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 IgG antibody-effector functions generated during dengue infection: an antibody-dependent enhancement assay, an 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	2017-2025
Collaborations	Virology Unit, IP du Cambodge (DUONG V), Rockefeller University (RAVETCH J, BOURNAZOS S), Antwerp University (ARIEN K)
Funding	NIH PICREID (1U01AI151758 – 01): 2020-2025 Calmette-Yersin Pasteur Network Postdoctoral Research Grant 2023-2024

Aim 1.3 Understand the Role of Different CD4+ T Cell Subsets in the Progression of DENV Infection

The contribution of CD4+ T cells in protection from disease or the development of immunopathogenesis after dengue infection is unclear, however, recent data has pointed towards CD4+ T cells having a protective role during infection. By immunoprofiling of asymptomatic individuals, our previous work has shown an association between proliferation and activation of the $CD4^+$ T cell compartment with asymptomatic outcome of infection, irrespective of previous dengue infection or viral load (Simon-Loriere, Duong et al., Sci Transl Med, 2017). Regulatory T cells (Tregs) control unwanted immune responses like autoimmune reactions. Tregs subsets and functions are understudied during dengue infection. We hypothesize that defects in the regulatory T cell compartment contribute to the immunopathology of dengue infection as observed in severe dengue. Hence, we aim to understand the function of regulatory T cells during the course of dengue infection in mild and severe dengue cases. For this project, all experiments have been performed, and we will focus on data analysis and manuscript writing for the year 2023.

Start/End Year	2017-2023
Collaborations	University of Hasselt, Belgium (KLEINEWIETFELD M)
Funding	HHMI/Wellcome International Research Scholars Program (208710/Z/17/Z). 2017-2022

Aim 1.4 Immunity to Aedes Mosquito Saliva

To extend our observations in humans (see above, major achievements), we aim to investigate the effect of mosquito saliva on the immune response to dengue. We will compare dengue replication in primary immune cells (dendritic cells and keratinocytes) in presence or absence of *Aedes aegypti* salivary gland homogenate and we will identify changes in immune responses of primary immune cells (dendritic cells and keratinocytes) to dengue in the presence of *Aedes aegypti* saliva.

Start/End Year	2020-2023
Collaborations	Entomology Unit, IP Cambodia (BOYER S), Laboratory of Malaria and Vector Research, NIAID, NIH (MANNING J, OLIVEIRA F), MiVEGEC Unit, IRD, UR224 (MISSE D)
Funding	Calmette-Yersin RIIP PhD program: 2020-2023

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

IP du Cambodge is part of the NIH-funded consortium PICREID. Here, the Immunology Unit is involved in aim 4, the study of hosts' adaptive immune responses to emerging infectious diseases in South-East Asia. In this framework, we have established a single cell analysis platform at *IP du Cambodge*. We will increase our insight into the adaptive immune response (Both B and CD T cell responses) at a single-cell level and the sequence–function relationship of human antibodies generated during arbovirus infections, by combining sequencing at a single-cell level with antibody repertoire analysis. We will study function and characterize structure at a single-antibody level. Moreover, we aim to perform detailed immunoprofiling of flavivirus encephalitis. We will provide a novel understanding of the role of cellular immunity in DENV disease. The proposed activity will allow the implementation of infrastructure and an analysis pipeline for outbreak preparedness in areas where viruses with potential pandemic threats circulate.

Start/End Year	2020-2025
Collaborations	Virology Unit, IP du Cambodge (DUONG V), Epidemiology and Public Health Unit, IP du Cambdoge (LY S), Entomology Unit, IP du Cambodge (BOYER S), Functional Genetics of Infectious Diseases Unit (SAKUNTABHAI A)
Funding	NIH PICREID (1U01AI151758 – 01): 2020-2025

Axis 2: Biomarkers of Infectious Diseases

Aim 2.1 MicroRNA as Potential Biomarkers of HIV/IRIS

We follow up on the project ANRS no. 12358, 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 were submitted for evaluation to DARRI and *Institut Carnot Pasteur MS.*

Start/End Year	2022-2023
Collaborations	IP Paris (SCOTT D), IP du Cambodge (PEAN P), IP du Cambodge (BORAND L)
Funding	DARRI, IP Paris, France: 2022-2023

Aim 2.2 MicroRNA as Potential Biomarkers of Infectious Diseases

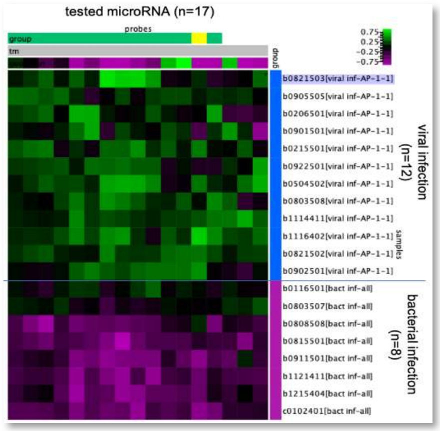


Figure 12. Heatmap displays the expression levels of serum microRNA (fold change MFI) in viral and bacterial infection.

MiRNAs have been identified in numerous diseases, particularly in cancer, which presents them as potential novel diagnostic and prognostic biomarkers with high specificity and sensitivity. The detectable miRNAs in body fluids and tissue with high stability provide an abundant source for miRNA-based biomarkers. The microRNAs detection in biofluid by flow cytometry technique, which has been validated and 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 2023 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 virus-infected children versus respiratory bacteria-infected (figure 4.4.). Collaboration with Dr. Karlsson (IPC, Virology Unit). Next, proposal to internal fund or AMR consortia fund in 2023) (5) biomarkers of Schistosomiasis infection.

Start/End Year	2023-2025
Collaborations	IP Rome (SCAGNOLARI C), Virology Unit, IP du Cambodge (KARLSSON E), CNM/MoH (VIRAK K)
Funding	To be identified

Axis 3: Vaccine Responses to Rabies Virus Vaccination

We are establishing a biobank for the study. All samples and viral strains collected in this biobank will be used for further in-depth analysis of immune responses such as T cell responses and antibody affinity responses. Patient recruitment is continuing since 2020.

Start/End Year	2020-2023
Collaborations	Virology Unit, IP du Cambodge (DUONG V), Epidemiology and Public Health Unit, IP du Cambodge (LY S / BORAND L), Vaccination center, IP du Cambodge (PENG Y)
Funding	Internal IPC project: 2020-2023

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 the flow cytometry technique for cell lineage identification in acute leukemia and chronic lymphoproliferative disorders (CLPD) in pediatric patients (n=100). We use IVD BD^{TM} ALOT and LST commercial kits for cell lineage assessment and clonotype determination, respectively. Data acquisition and analysis is performed using BD FACS Canto II flow cytometer. The specimens are bone marrow aspirate (BMA) and peripheral venous blood (PB) of sick children admitted in Kantha Bopha hospital. Since March 2022, BMA and PB specimens of 26 children suspected acute leukemia and CLPD are analyzed (e.g. 38 test in total). The majority of suspected acute leukemia specimens are associated with B cell lineage (n=19) followed by mixed phenotypes (T/AML or B/AML) (e.g. unidentified cells lineage) (n=15) and myeloid lineage (n=4). In several specimens subjected to CLPD testing, we did not identify any aberrant mature lymphoid cells or restrictive B cell or T cells clones. These patients might require an assessment of lymph nodes by fine needle aspiration or tissue biopsy.

Start/End Year	2022-2023
Collaborations	Medical Biology Laboratory, IP du Cambodge (GUILLARD B), Kuntha Bopha Children Hospital (LAURENT D)
Funding	Internal IPC project: 2022-2023

4.3.4 Support to National Authorities

Tineke Cantaert, PhD and Pean Polidy, MD, PhD are both members of the steering committee for the International Master's Degree Program on Infectiology and coordinators of the Immunology Module in year 1 of the Master's Degree. 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

Students

PhD students:

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

Internship/Master students:

- Chheng Meng An: Royal University of Phnom Penh (2022), Bachelor thesis: *Response of Regulatory T Cells to Dengue Infection*
- Hugo Brouque: Grenoble Alpes University (2022), Master 1 Internship, *Adaptive Memory Responses to Arbovirus Infection.* Supported by *Fondation Pierre Ledoux – Jeunesse Internationale*
- Karolina Johanna Svedlund: University Paris-Saclay (2022), Master 1 Internship, *T-Cell response to rabies vaccination.*

- Ya Nisa: University Paris-Saclay, Phnom Penh (2022), Master 1 Internship, *Study of Immune Responses to Plasmodium Vivax Infection.*
- Hak Rith Thea: AgroParisTech, France (2022-2023), Engineering Internship, *Investigation of Adaptive Immune Responses after Dengue Virus Infection.*
- Eliott Benchinou: University Paris Saclay, Master 1 Internship, A *Set of Plasmatic MicroRNA as Predictive Biomarkers of IRIS in HIV/TB Coinfected Iatients*. Supported by *Fondation Pierre Ledoux – Jeunesse Internationale*

Teaching

- Tineke Cantaert, PhD and Pean Polidy, MD, PhD: Master's in Infectious Diseases, *Université Paris-Saclay*, University of Health Sciences, 10 hours (M1 and M2)
- Tineke Cantaert, PhD and Pean Polidy, PD, PhD: Member of the Steering Committee for the International Master's in Infectious Diseases and Coordinator of the Immunology Module (M1).
- Tineke Cantaert, PhD: Master's in Immunology-ImmunoPathology, Sorbonne University, 2 hours (M2)

4.3.6 Outlook

The strengths of the Immunology Unit in research on infectious diseases 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. In 2022, our laboratory and office spaces have doubled, allowing us to expand our activities. We hired a project manager to facilitate all multi-partner collaborations in a timely fashion. One Cambodian student was enrolled in a PhD program (University of Antwerp). 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. For example, two PhD students of the Unit will graduate in 2023, and a hands-on 6-day course in flow cytometry and its applications is planned for September 2023, where we aim to attract international students.

Next year, we will attract a skilled bioinformatician who will increase our capacity for -omics and big dataset analysis, and who is committed to transfer his knowledge to Cambodian scientists. In 2023, we aim to formalize a first collaborative project with the University of Health Sciences to investigate the prevalence and immune mechanisms of type II mediated diseases. We also aim to apply for 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 2022, multiple innovative grant proposals have been submitted, aiming to 1) secure our current research lines (axes 1-3), and 2) expand our research to new subjects with novel collaborating partners (e.g., parasite immunology, other arbovirus infections). This will guide our research programs for the upcoming 5 years.

4.3.7 Publications List in 2022

NOTE

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

1. **Rapid Generation of In-House Serological Assays Is Comparable to Commercial Kits Critical for Early Response to Pandemics: A Case With SARS-CoV-2.**
   Heidi Auerswald, Chanreaksmey Eng, Sokchea Lay, Saraden In, Sokchea Eng, Hoa Thi My Vo, Charya Sith, Sokleaph Cheng, Gauthier Delvallez, Vann Mich, Ngy Meng, Ly Sovann, Kraing Sidonn, Jessica Vanhomwegen, Tineke Cantaert, Philippe Dussart, Veasna Duong, Erik A. Karlsson.
   Front Med (Lausanne). 2022;9:864972. Epub 2022/05/24. DOI: 10.3389/fmed.2022.864972. PubMed PMID: 35602487; PMCID: PMC9121123.

2. **Evaluation of cutaneous immune response in a controlled human in vivo model of mosquito bites.**
   David Guerrero, Hoa Thi My Vo, Chanthap Lon, Jennifer A. Bohl, Sreynik Nhik, Sophana Chea, Somnang Man, Sokunthea Sreng, Andrea R. Pacheco, Sokna Ly, Rathanak Sath, Sokchea Lay, Dorothée Missé, Rekol Huy, Rithea Leang, Hok Kry, Jesus G. Valenzuela, Fabiano Oliveira, Tineke Cantaert**, Jessica E. Manning**.
   Nat Commun. 2022;13(1):7036. Epub 2022/11/19. DOI: 10.1038/s41467-022-34534-9. PubMed PMID: 36396947; PMCID: PMC9672097.

3. **Temporal patterns of functional anti-dengue antibodies in dengue infected individuals with different disease outcome or infection history.**
   Hoa Thi My Vo, Vinit Upasani, Heidi Auerswald, Sokchea Lay, Sotheary Sann, Axelle Vanderlinden, Sreymom Ken, Sopheak Sorn, Sowath Ly, Veasna Duong, Philippe Dussart, Tineke Cantaert.
   Sci Rep. 2022;12(1):17863. Epub 2022/10/26. DOI: 10.1038/s41598-022-21722-2. PubMed PMID: 36284116; PMCID: PMC9596418.

4. **A Novel AICDA Splice-Site Mutation in Two Siblings with HIGM2 Permits Somatic Hypermutation but Abrogates Mutational Targeting.**
   Johannes Dirks, Gabriele Haase, Tineke Cantaert, Lea Frey, Moritz Klaas, Christian H. Rickert, Hermann Girschick, Eric Meffre, Henner Morbach.
   J Clin Immunol. 2022. Epub 2022/03/06. DOI: 10.1007/s10875-022-01233-5. PubMed PMID: 35246784.

5. **MicroRNAs as predictive biomarkers of immune reconstitution inflammatory syndrome.**
   Patent No NT/ NG/IDM-22-0055. Scott D, Pean P, Borand L, Meng R, Bunnet D, Madec Y

6. **Editorial: Balanced and Unbalanced Immune Response to Dengue Virus in Disease Protection and Pathogenesis.**
   Tineke Cantaert, Nolwenn Jouvenet, Sean A. Diehl.
   Front Immunol. 2022;13:835731. DOI: 10.3389/fimmu.2022.835731. PMID: 35222425;

7. **Robust and Functional Immune Memory Up to 9 Months After SARS-CoV-2 Infection: A Southeast Asian Longitudinal Cohort.**

   Hoa Thi My Vo*, Alvino Maestri*, Heidi Auerswald, Sopheak Sorn, Sokchea Lay, Heng Seng, Sotheary Sann, Nisa Ya, Polidy Pean, Philippe Dussart, Olivier Schwartz, Sovann Ly, Timothée Bruel, Sowath Ly, Veasna Duong, Erik A. Karlsson**, Tineke Cantaert**.

   Front Immunol. 2022;13:817905. DOI: 10.3389/fimmu.2022.817905. PMID: 35185909; *co-last authors

4.4 Virology Unit

4.4.1 Functional Structure

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), 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 comprises 48 staff including seven PhDs, two PhD candidates, four master's degree holders, two medical doctors and two pharmacists. In 2022, the Unit experienced the departure of scientists, technical staff and students. Dr. Cécile Troupin and Dr Narjis Boucli had left IPC as well as two technical staff.

Within the four components, including research and surveillance, 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, and 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 hosts 2 national reference centres for the diagnostic of arboviruses and rabies, and 3 international reference laboratories, including the National Influenza Center, the WHO's Regional H5 Reference Laboratory, and a WHO Global COVID-19 Reference Laboratory.

4.4.2 Research Programs – Major Achievements in 2022

Axis 1: Arboviral Diseases

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 Immunology Unit section. 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 2023, a total of 525 suspected dengue cases were included, and 134 were positive for dengue infection (RT-PCR confirmation). The majority of the samples came from Baray-Santuk and Kampong Thom hospitals (Figure 13). Following the household investigation around the index cases that started on 1 November 2022, we included 1280 participants that are currently investigated serologically for past arbovirus infections.

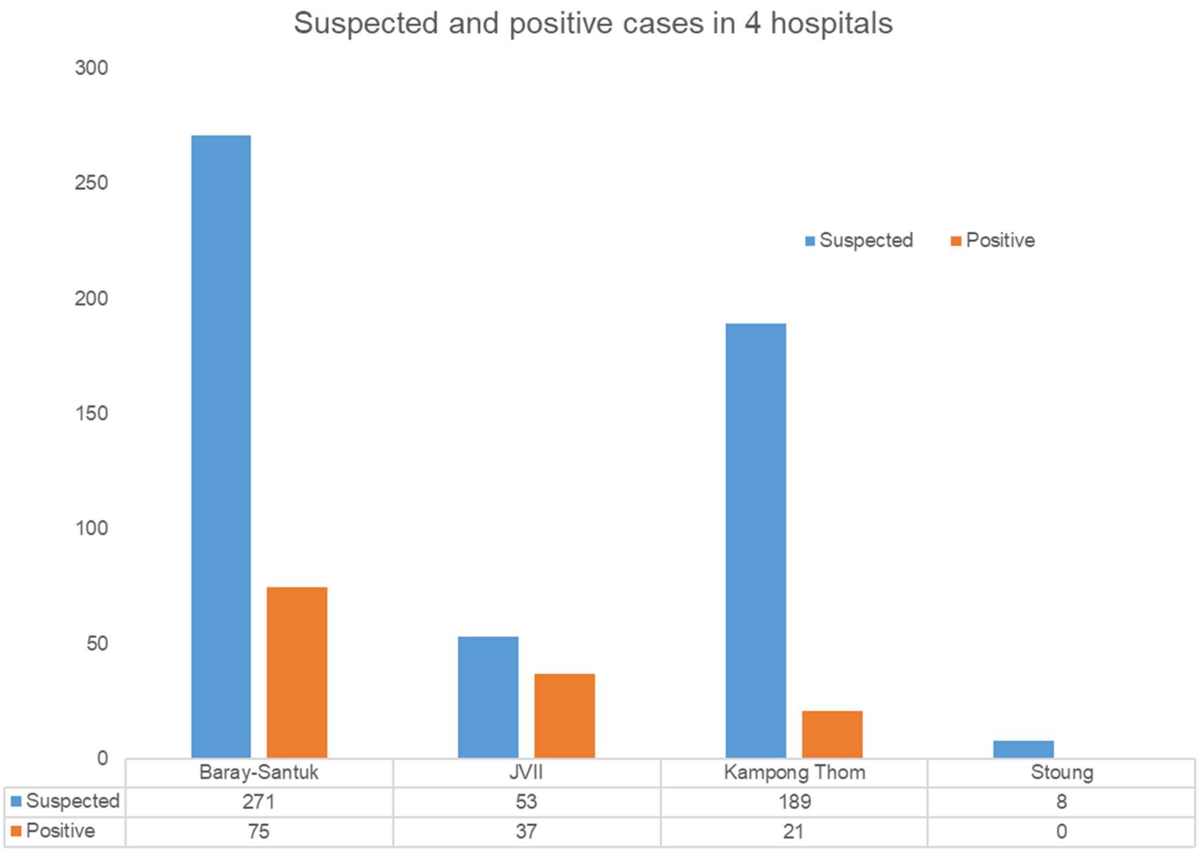


Figure 13. Total cases of confirmed and suspected dengue infection from four hospitals

Collaborations	Institut Pasteur du Cambodge: Virology Unit (V. Duong, S. Ken, P. Ou, L. Heng and O. Yanneth), Epidemiology and Public Health Unit (S. Ly), Immunology Unit (T. Cantaert), and Medical and Veterinary Entomology Unit (S. Boyer) IP, Paris (Anavaj SAKUNTABHAI)
Funding	NIH collaborative agreement (1-IPC-NIH-U01-AS-2020): August 2020 to May 2025

Description of the intra-subject time course of viraemia during the acute phase of chikungunya infection, of the risks of chikungunya transmission and of the immune response characteristics among members of a single household in Cambodia.

Chikungunya is a viral disease transmitted to humans by infected mosquitoes. It is caused by the chikungunya virus (CHIKV). There is currently no vaccine or specific drug against the virus. The unpredictability of a chikungunya outbreak, combined with its frequently short duration, pose considerable difficulties in initiating a trial before the risk of infection in the community has fallen to low levels. Hence, we lack a comprehensive understanding of the duration of CHIKV viraemia in patients, and of the risk of transmission in a household setting. This information would help improve the clinical feasibility evaluation of potential new treatments. The main objectives were to characterize kinetic viraemia during the acute phase of CHIKV infection, and to determine the risk of CHIKV transmission between household members of an index case.

The study was carried out in Kampong Thom Province. It began in December 2020 and concluded October 2022. Patients were recruited at Kampong Thom's Provincial Referral Hospital and at two other district referral hospitals in Baray and Staung Districts, Kampong Thom Province. Later, the study was expanded countrywide due to a reduction in new cases reported in these two provinces. Therefore, Jayavarman VII Hospital in Siem Reap and Kuntha Bopha Hospital in Phnom Penh were included to increase sample availability. Household members of CHIKV confirmed cases were also enrolled. Blood samples were collected for virological tests (viral detection by RT-PCR and IgM/IgG detection by ELISA assays). In total, we were able to recruit 29 index cases (14 adults > 18 years old and 15 children, > 2 years old and < 18 years old). The peak viral load ranged from 1.091×10^3 to 8.233×10^9 and the peak was observed on average 2.7 ± 3.2 days after the onset of symptoms. IgM and IgG became detectable on average 5.4 ± 2.2 days and 8.5 ± 2.1 days after symptom onset, respectively. Unfortunately, we were only able to include 147 family members. Estimates of the secondary chikungunya attack rate within households ranged from 12% to 25%. Data processing is in progress.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (V. Duong, S. Ken, P. Ou, L. Heng and O. Yanneth); Epidemiology-Public Health unit (S. Ly, S. Sorn, K. Nguon); Immunology unit (T. Cantaert) Evotec (Hugh Watson)
Funding	Evotec International GmbH: December 2020 to October 2022

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), and we again aimed to collect 5,120 poultry samples from 2,560 birds (paired tracheal and cloacal swabs) over the 2021–2022 period. In 2021, we were able to collect 2,560 samples from 1,280 birds, with an overall positivity rate of 25 %. In 2022, this collection continued in the beginning and late parts of the year, with 2,911 samples collected from 1,280 birds, and 351 environmental samples (see below).

The collection, analysis, and sequencing are complete for the 2017–2022 sessions, and several manuscripts are in preparation (see below). This collaboration is planned to continue for the 2023 season.

Sequencing of avian influenza samples to investigate outbreaks and the diversity of influenza viruses circulating in Cambodian poultry.

We have been conducting LBM surveillance in the Orussey Market in Phnom Penh since 2011. This is intended to determine the circulation characteristics of avian influenza in Cambodia. Isolates from 2015 to 2019 have been transferred to the WHOCC in Melbourne for full genome sequencing using NGS and have been completed. These analyses will reveal important information about the rate of reassortant events occurring in LBMs and the risk of emergence of novel AIV strains. Final samples from 2022 have been 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. In 2022, we were able to sequence 326 AIV samples, 160 of these from 2022 surveillance.

Detection of A/H5 clade 2.3.4.4b viruses in Cambodian live bird markets.

In recent years, A/H5Nx clade 2.3.4.4b highly pathogenic avian influenza viruses (HPAIv) have spread globally, causing severe outbreaks in Africa, Europe, Asia, and most recently, North America. In addition to a number of LPAIv, multiple A/H5 subtypes and clades continue to circulate in Cambodia. H5N1 clade 2.3.2.1c viruses were still detected regularly in 2022. H5N6 clade 2.3.4.4g and 2.3.4.4h viruses were identified in Cambodian LBMs from late 2018 through 2020. During active surveillance in late 2021, duck samples from Phnom Penh, Kandal and Takeo markets tested positive for the H5 HA gene but were negative for neuraminidase (NA) subtype N1 by real time RT-PCR. Further analysis revealed these samples to be subtype H5N8, and further characterization by full genome sequencing confirmed that these samples were positive for HPAI H5N8 and found that they corresponded to H5 clade 2.3.4.4b (Figure 14). The detection of these novel lineage viruses, their association with outbreaks in poultry throughout the world, their zoonotic potential, co-circulation with other AIV of concern, and the ability of these A/H5Nx viruses to evolve rapidly make it imperative to maintain constant, rigorous, and vigilant surveillance for AIV in Cambodian poultry populations. 2.3.4.4b viruses continue to be detected into 2022.

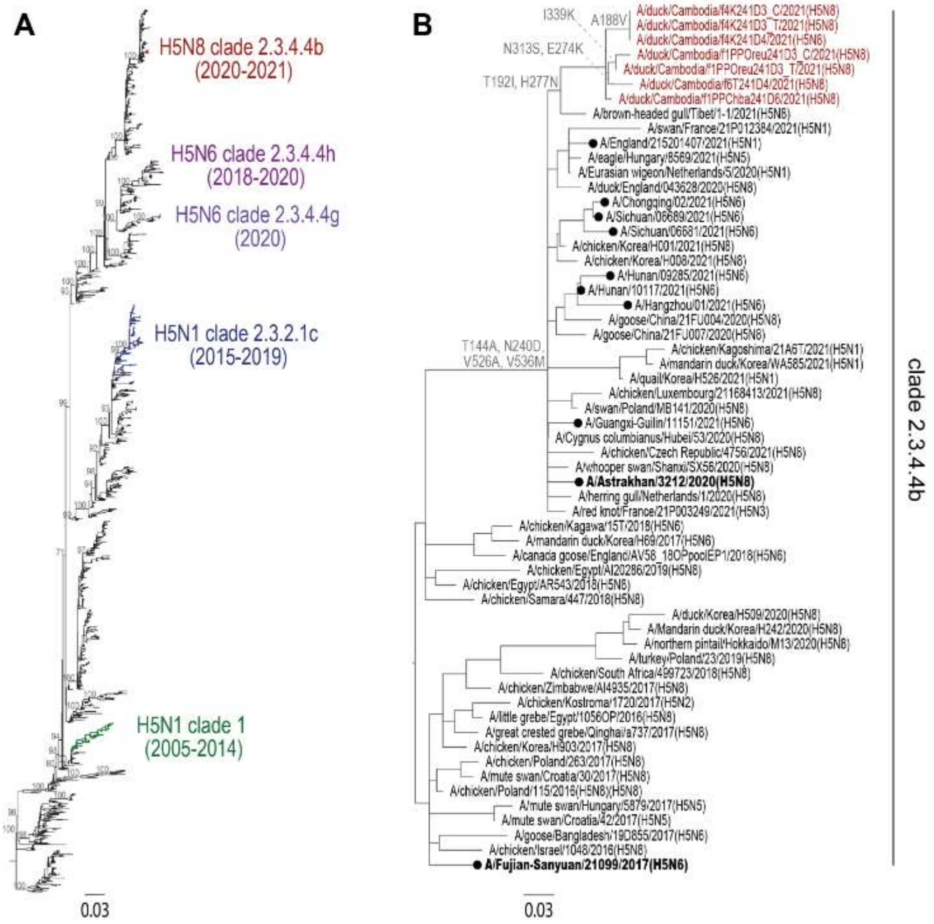


Figure 14. Maximum likelihood phylogeny of the HA genes of H5 viruses in Cambodia.
Scale bars represent nucleotide substitutions per site. A, All HPAI H5Nx clades detected in Cambodia. B, H5N8 clade 2.3.4.4b. Candidate vaccine viruses for clade 2.3.4.4b are shown in bold. Amino acid mutations are indicated at select nodes. Tip shapes indicate cases of human infection with avian H5Nx viruses

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 2020–2022 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 2023.

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.

A human case of avian influenza A(H9N2) virus infection in Cambodia, 2022.

One sample was collected at Angkor Children's Hospital from a 1 year and 1 month old female ILI case from Trapaing Roun Village, Roesei Lok Commune, Chi Kreng district, Siem Reap province on 4 March 2022. The patient had a slight fever (>38°C), a cough, a sore throat, a runny nose and difficulty breathing. The sample was tested at NIPH on 5 March 2022, and identified as influenza A, subtype A/H9N2 on 8 March 2022. The sample was then transferred to the Virorogy Unit at Institut Pasteur du Cambodge (IPC) on 9 March 2022 and was tested for Influenza types A and B, as well as for haemagglutinin (HA) subtypes of human seasonal A/pdmH1 and A/H3, and avian subtypes A/H5, A/H7, and A/H9. RNA was extracted from the sample and was analyzed via standard RT-qPCR assays. The sample was confirmed ***positive* for *Influenza A(H9N2)*** and negative for all other tests performed. Re-extraction and retesting of the sample yielded equivalent results. A(H9N2) virus was also detected from a chicken at the infected child's house. Subsequent sequence analysis revealed genetic proximity between the HAs of the human and chicken viruses which supports a possible link to a recent shared "ancestor", suggesting that the source of the child's exposure was from household chickens (Figure 15). The pandemic potential of these viruses remains to be assessed.

The A/H9 subtype, a low-pathogenicity avian influenza virus (LPAIV), was isolated for the first time in Wisconsin, USA in 1966 in turkey flocks. Since the early 1990s, the virus has spread extensively among poultry populations, including in Cambodia. 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. Last case was reported with date of onset of 28 January 2022 in China.

This is the second human A(H9N2) detection in Cambodia. Previously, one A/H9N2 case had been detected in Cambodia on 4 March 2021, in a 3-year-old boy living in Thnal Bale Village, Ampil Commune, Bakong District, Siem Reap Province.

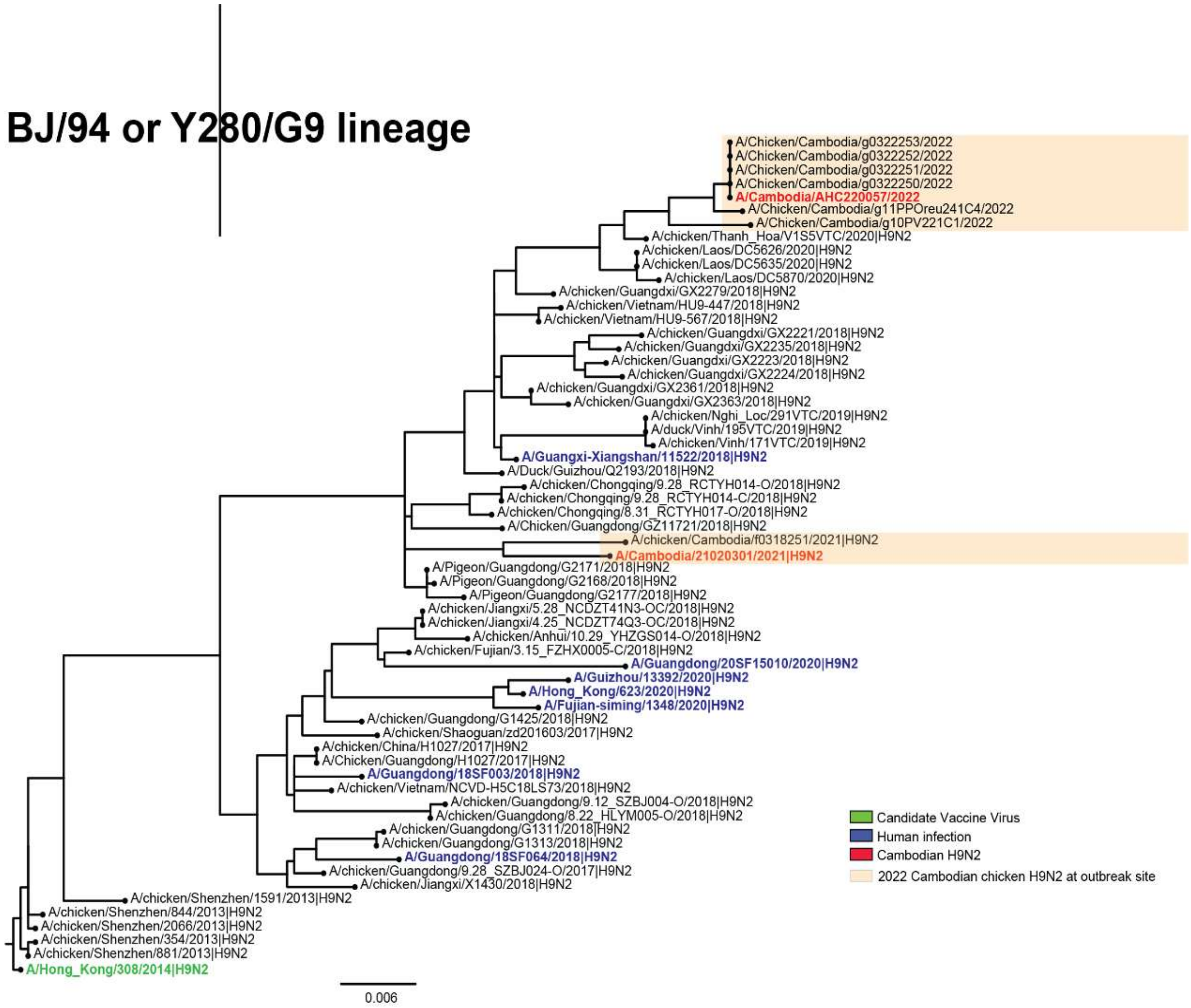


Figure 15. Maximum likelihood phylogeny of the HA genes from A/H9 cases and household samples from chickens in Cambodia, 2021-2022. Scale bars represent nucleotide substitutions per site.

Longitudinal serological surveillance for AIV infection in poultry workers.

Following a serological survey in 2015, and in line with what was done in the 2017-2018 surveillance, longitudinal human serum sampling was conducted twice in 2018 (once at the start of the study, once between the Pchum Ben and Bon Om Touk holidays) and twice in 2019 (between Chinese New Year and Khmer New Year, and at the end of the study) by the epidemiology unit at IPC, in conjunction with NAHPRI in four provinces. In 2020, four more sessions were planned for collection in the same locations; however, due to issues surrounding the COVID-19 pandemic, only 2 sessions were carried out. Four further sessions were planned for 2021–2022; however, again due to issues surrounding the COVID-19 pandemic, no samples were collected in 2021, but samples were collected at the end of 2022, and continued collection is planned into 2023.

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, and we were able to collect 1647 swine samples, with collection continuing into 2023. No influenza viruses have been detected in swine samples in 2022.

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

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 and 2022 samples have also begun at IPC. Analysis of the etiology database was completed in 2022 and we are actively collaborating with epidemiologists and biostatisticians for the best way to analyze and present the data. A manuscript describing the 2014–2022 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-2023.

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
Funding	US-DHHS, USAID, FAO, WHO, Virology internal funding: 2014-2022

Surveillance of avian influenza and identification of hotspots of spillover between poultry and wild birds.

The recent emergence of H7N9 in China, the emergence and global spread of H5Nx clade 2.3.4.4. and continual H5N1 outbreaks in domestic poultry highlight the need to understand the prevalence as well as genetic and phenotypic diversity of avian influenza viruses circulating in wild bird reservoirs in Southeast Asia. While countries in SEA, especially Cambodia, have a high prevalence of AIV in poultry, very little is known about the prevalence in wild birds. Therefore, in 2019, in collaboration with the Wildlife Conservation Society (WCS), NAHPRI and other international partners we have started a project looking at influenza prevalence in wild birds in the Mekong Delta region to gain an understanding of basic prevalence and to identify potential hotspots of spillover from wild populations to domestic poultry. Funding for this study was approved in November 2021 and sampling continued into 2022 at 3 locations in 12 field missions. A total of 2702 samples were collected from wild birds and their surroundings, including faeces (n=2,228), water (n=168), sediment (n=306). No samples were positive for AIV in routine surveillance. A total of 4449 samples were collected from domestic poultry located near sampled wild birds, and included predominantly chickens, domestic ducks, and Muscovy ducks. Some samples from geese, and one set of poultry slaughterhouse samples were collected. A total of 36 samples from domestic poultry were found to contain AIVs. Interestingly, during this surveillance at the wild bird–poultry interface, three viruses (February 2022: n=1, March 2022: n=2) were identified as H14N2, originating from one semi-free ranging duck farm. These samples are currently undergoing further analysis and publication is expected in mid-2023.

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

Avian and human influenza surveillance activities in Cambodia and surveillance 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 2022, we were able to collect 351 ES for comparison to individual bird samples. Sampling continues into 2023 and analysis is ongoing.

Collaborations	Institut Pasteur du Cambodge : Virology unit (E. Karlsson) Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute
Funding	Food and Agriculture Organization of the United Nations, 2022-2023

Axis 3: Zoonoses

HEPAR: Rodents as reservoirs for hepatitis E Virus (HEV-C), arenavirus and other rodent-borne viruses and risk assessment of infection in humans in Cambodia.

The study's objective remains to investigate HEV, arenavirus and other rodent-borne virus infections in rodents, and to estimate the seroprevalence of those rodent-borne virus infections in humans in both urban and rural environments.

A cross-sectional prospective study in selected open markets of the capital Phnom Penh and rural villages in Kampong Cham and Preah Sihanouk Provinces was carried out. Rodent trapping sessions and blood sampling, and data collection on humans exposed to rodents were conducted during rainy and dry seasons over a two-year period (June 2020–April 2022). In total, 750 rodents were trapped (336 and 414 during the rainy and dry season, respectively). Four genera were morphologically identified (*Rattus*, *Bandicota*, *Suncus* and *Mus*). 788 participants were enrolled in the three provinces and 70.4 % (555/788) were monitored up to two years after their inclusion.

Various pools of organs have been tested for HEV-C, HEV-A and ARV using molecular detection methods. HEV-C was detected and confirmed by Sanger sequencing in 14 specimens (1.9%). HEV-C were detected in *R. norvegicus* (n=14) and all positive rodents were from Phnom Penh markets. ARV was detected in 39 specimens (5.2%), of which seven were detected in Phnom Penh markets, five in Kampong Cham sites, and 27 in Preah Sihanouk sites. Twenty-nine were detected among *R. exulans*, seven in *R. norvegicus* and three in *R. rattus*. Sequencing of PCR products identified them as Cardamones virus.

In total, 788 individuals working in markets in Phnom Penh, and living in communities where rodents were captured during the first session in the wet season of 2020, were enrolled in the study. Follow-up sampling sessions were implemented two years later, in 2022. Commercial ELISA kits for the detection of antibodies (IgG) against HEV (Wantai BioPharm) allowed the confirmation of exposure to HEV in 191 participants (24.2 %): 125/304 (41.1 %), 23/196 (11.7 %) and 43/288 (14.9 %) in Phnom Penh, Kampong Cham and Sihanouk sites, respectively. An in-house ELISA for IgG targeting arenavirus nucleoprotein (NP), Cardamone's variant of Wenzhou Virus (WENV), was used to screen human serum samples. Out of 788 serum samples, 100 (12.7 %) were positive for IgG antibodies, with a higher percentage in urban areas (Phnom Penh; 21.7 %) compared to other sites (Kampong Cham; 9.2 % and Preah Sihanouk; 5.6 %).

The whole genome sequencing of positive samples is being carried out using metagenomics and AmpliSeq sequencing techniques. The development of a seroneutralization assay for confirmation of positive samples by ELISA is ongoing.

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

PICREID: Hantavirus detection and characterization in rodents and human seroprevalence study in Cambodia.

Hantaviruses are widespread across the world. In Asia, there is a lot of evidence of the circulation of these viruses in the eastern part of the continent (especially in Far-East Russia, China, and Republic of Korea). In Cambodia, we detected the presence of the Seoul orthohantavirus (SEOV) and Thailand hantavirus (THAIV) for the first time in 1998. SEOV was detected in brown rats (*Rattus norvegicus*) sampled in Phnom Penh and THAIV in black rats (*Rattus rattus*) collected in rice fields in neighboring villages. Very little data regarding serological evidence of infection in humans is available in Cambodia.

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

The presence of hantavirus was detected in 25/750 (3.3 %) of all rodents captured in Phnom Penh markets only. Analysis of partial hantavirus L gene sequences revealed that 24 rodents were infected with SEOV, one rodent with Thottapalayam virus. In collaboration with IP Paris, the whole genome sequences of SEOV were obtained from the 24 rodents using NGS Oxford nanopore technology. In humans, the initial seroprevalence of anti-hantavirus IgG was 10.0 % (79/788) in 2020. A second serum sample was available in 2022 for 555 individuals (70.4 %). Six (1.1 %) out of 555 individuals became anti-hantavirus IgG positive. Of the six sero-converted individuals, one tested positive for anti-hantavirus IgM. The hantavirus seroprevalence was significantly higher in urban areas than in rural ones (13.8 % vs. 8.9 %, p=0.03).

Collaborations	Institut Pasteur du Cambodge: Virology Unit (J. NOUHIN, V. HUL, J. GUILLEBAUD, S. KEN, L. KHUN, R. LIM, H. AUERSWALD, and V. DUONG) Institut Pasteur Paris: French National Reference Center for Hantaviruses (J.M. REYNES); Urgent Response to Biological Threats laboratory (J.C. MANUGUERA, J. VANHOMWEGEN and V. CARO) and A. SAKUNTBHAI
Funding	CREID Network Pilot Research Program (7-312-0217530-66440L under NIAID Award Number 1U01AI151378-01): May 2021 – April 2022

ZooCoV: Toward an integrated surveillance of potential zoonotic beta coronaviruses in the wild animal value chains of Cambodia.

Beta-CoVs were responsible for three major respiratory infection outbreaks worldwide over the last two decades, including the current COVID-19 one, involving viral spillover at the human/animal interface. Data on animal reservoirs, as well as on cultural, sociological, and ecological factors driving Beta-CoVs spread to humans and emergence is limited. The study objectives were to 1) provide new knowledge on wild meat trade chains in Cambodia, 2) document the diversity of Beta-CoVs circulating through these chains, 3) describe and understand the practices and perceptions regarding the bushmeat trade and consumption at the human/wildlife interface, and 4) develop a flexible and integrated early-detection system of viral spillover events.

The virology unit is responsible for WP3 “risk quantification at the human/wildlife interface”. Activities are focused in two pilot provinces—Mondulkiri and Stung Treng—where various study sites were selected to conduct a wildlife and bushmeat trade survey in the human population using quantitative surveys during the rainy and dry seasons in 2020 and 2021. All individual serums (n=901) have been tested for the detection of SARS-CoV-2 antibodies using in-house ELISAs and Foci Reduction Neutralization Tests (FRNT). Follow-up survey was carried out among 189 participants (second serum sample and additional questionnaire). No sample was detected with anti-SARS-CoV-2 IgG, however, 265 displayed a positive or equivocal result for CoV antibodies. Luminex technology was used to characterize the immune response of participants to various CoVs (in collaboration with the TRANSVIHMI unit, IRD), including SARS-CoV, MERS-CoV, SARS-CoV-2, and four seasonal CoVs (OC43, HKU1, NL63 and 229E). Preliminary data is available, and analyses are ongoing as of this report’s publication.

Concurrent to human surveys, wildlife sampling was implemented in both provinces, either with active sessions (bats and rodents sampling), opportunistic sessions, or through collaborations with conservation NGOs. The following results include sampling and testing from a previous twin project, RhinoKhoV, funded by MUSE-16297. In total, 2723 individuals/specimens from bats, rodents and other mammals were tested for the presence of CoV using molecular techniques (real-time PCR for SARS-CoV targeting E and N genes, conventional PCR for Pan-CoV detection). Overall, 55 specimens (2.0 %) tested positive, and results were confirmed by sequencing for CoVs. The prevalence was at 3.4 % (29/865) in individual bats, 2.5 % in rodents (4/159), 1.5 % (21/1,360) in bat faeces, and 0.3 % (1/298) among other wildlife’s faeces. Both alpha-CoV and Beta-CoV were detected, and nine SARS-CoV-2-related viruses were detected in three different bat species. Analysis is ongoing to further characterize these viruses at the time of this report.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (DUONG Veasna, Julia GUILLEBAUD, YANNETH Oudamdaniel, HUL Vibol and HOEM Thavry); Epidemiology-Public Health Unit (LY Sowath) CIRAD (V. CHEVALIER); IRD (Martin PEETERS); Wildlife Conservation Society; Flora and Fauna International; Jahoo NGO Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute; Forestry Administration Ministry of Health: Cambodian CDC
Funding	ANR, Région Occitanie, Pasteur Asia Foundation: June 2020 – January 2022

Benchmarking novel environmental surveillance in bat caves and other high-risk areas for expanded early-warning surveillance.

In Cambodia, current bat 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.

Seven provinces were selected for sampling sessions according to the presence of bats caves and/or known bats roosts. In each province, several sites have been visited at least one time for specimen collection. Screening of viral families was performed on faeces, urine, air, and surface specimens. Aliquots of faeces and urine were combined in groups of five before analysis. All other specimens were tested individually. Comparison of air sampling and PCR detection techniques for these samples is ongoing, and a publication is expected by mid-2023.

Collaborations	Institut Pasteur du Cambodge: Virology unit (Erik KARLSSON, DUONG Veasna, Julia GUILLEBAUD, YANNETH Oudamdaniel, HUL Vibol and HOEM Thavry); Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute; Forestry Administration
Funding	Food and Agriculture Organization of the United Nations: May 2022 – September 2022

In the air tonight, environmental sampling and viral metagenomics: a tool to expand and enhance genomic surveillance at high-risk human-animal intrefaces?

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 analyzed 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.

Collaborations	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
Funding	NIAID Centers for Research in Emerging Infectious Diseases – EID-SEARCH: August 2022 – April 2023

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 2022, 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 2023.

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

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 2022, further work was extended to other bat cell lines (e.g., TB1 Lu purchased from ECACC) and primary bat cells.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (Erik KARLSSON, Heidi AUERSWALD and DUONG Veasna) Duke-NUS Medical School Singapore (Gavin J.D. SMITH)
Funding	Virology Unit's internal funding: 2021-2022

Risk assessment of the impact of the COVID-19 pandemic on tour guides becoming poultry farmers and live bird sellers.

The COVID-19 pandemic has dealt a devastating blow to the tourism industry in Cambodia, especially in Siem Reap's Angkor Wat UNESCO World Heritage Center. Tourist arrivals to Cambodia were down by 98.1 % in 2020–2021 and only slowly started to recover in 2022. In Siem Reap, visitors to Angkor in Q3 2020 were down 98 % compared to the same time in 2019, dropping from 418,070 visitors in 2019 to only 8401 in 2020. Due to the pandemic, it is estimated that as many as 30,000 to 40,000 jobs have been lost in Siem Reap alone, based on the number of closed hotels, restaurants, bars, shops, and spas, as well as job losses for more than 6000 licensed tour guides operating in Siem Reap. In response to the need for income and sustenance, it is rumored that a number of tour guides have turned to small-scale farming for basic subsistence during the crisis, including chicken farming. Many of them have not received any formal training in zoonotic risk. This increase in poultry farming, especially for individuals who may lack training in good farming practices and preventative capabilities, may cause an increase in avian influenza virus risks and circulation. This semi-quantitative cohort study seeks to understand the impact of COVID-19 on tour guides (and farmers), the resultant food insecurity, and the knowledge, attitudes, and practices in biosafety and biosecurity with regard to avian influenza risk. A cross-sectional study was conducted between 2021–2022, involving 331 tour guides and 69 poultry farmers in Cambodia before and during the COVID-19 pandemic. Participants were administered a survey to assess food security, income, and general farming practices. Survey data evaluated the risk perception of avian influenza virus (AIV), antimicrobial resistance (AMR), and general biosecurity management implemented on these poultry farms. Overall, food security decreased for 80% of tour guides during the COVID-19 pandemic. Approximately 21 % of tour guides interviewed used backyard poultry farming to supplement loss of income and food insecurity during COVID-19, with significantly higher risk as compared to traditional poultry farmers. Agricultural intensification in Cambodia due to the COVID-19 pandemic has caused an influx of makeshift farms with limited biosecurity. Inadequate biosecurity measures in animal farms can facilitate spillover and contribute to future pandemics. Improved biosecurity and robust viral surveillance systems are critical for reducing spillover risks from backyard farms.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (Erik KARLSSON); Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute; Ministry of Tourism
Funding	Food and Agriculture Organization of the United Nations: October 2021 – April 2022

Axis 4: Rabies and other viruses

Immunogenicity assessment of subjects receiving rabies post-exposure prophylaxis in Cambodia.

This study aimed to compare different post-exposure prophylaxis (PEP) vaccination schemes in the real-life setting of individuals attending IPC's rabies vaccination center in Phnom Penh. The antibody kinetics in patients were evaluated following either the one-week, 3-dose intradermal regimen (ID IPC regimen) or the two-week, 4-dose intramuscular regimen (IM). Overall, 210 study participants remained in the study and the development of neutralizing antibodies (nAb) was measured via fluorescent antibody virus neutralization test (FAVNT). All subjects, except one in the IM group, seroconverted after receiving three vaccination doses (Figure 16). The additional dose in the IM regimen led to an additional increase of the antibodies. The cellular immune response of a subset of study participants was analysed for the measurement of certain cellular immune response parameters by the Immunology Unit. Overall, the shorter and dose-saving ID scheme proved suitable for inducing a comparable humoral and cellular immune response to the conventional IM regimen.

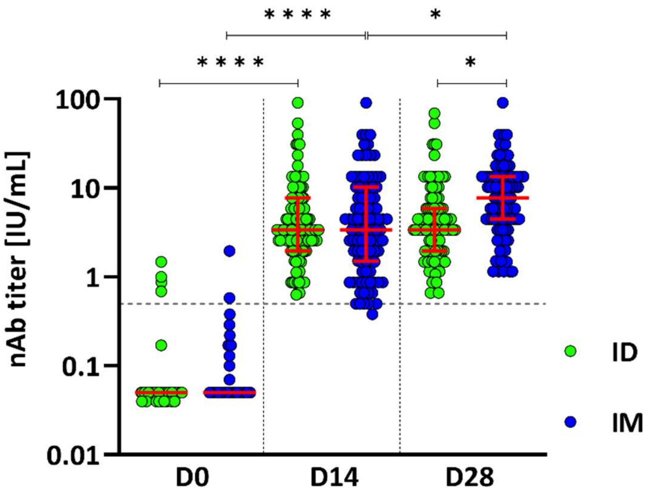


Figure 16. Rabies neutralization antibodies kinetic in patients after 3-dose intradermal regimen (ID, green) compared to 4-dose intramuscular regimen (IM, blue)

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

Analysis of long-term immunity induced by rabies vaccination in dogs.

A multi-partner rabies control program started in 2017 to reduce human rabies cases in two Cambodian peri-urban and rural provinces, namely Kandal and Battambang. The first achievements of this program were an estimation of the demographic parameters of the selected dog populations, a deep understanding of dog–human relationships and dog management practices, as well as the estimation of the annual bite incidence rate and associated risk factors (Chevalier *et al.*, 2021, PLOS ONE). Furthermore, the program aimed to design a dog vaccination strategy adapted to dog demographics in Cambodia and focused on vaccination-induced immunity. To achieve this goal, we used post-vaccination serological follow-ups of dogs accompanying the above-mentioned dog demography survey, and we applied Bayesian modeling to evaluate the individual duration of protection. We investigated the development and the persistence of anti-rabies nAbs in 351 dogs (determined by FAVNT) after primary and booster vaccination. In the investigated dog populations, 61 % of the primary vaccinated dogs retained a protective nAb level one year after their vaccination. In contrast, a booster vaccination one year after primary vaccination led to 95 % of the dog population being protected against rabies infection. Interestingly, 3 % of the tested dogs (n=7) had rabies nAb even before vaccination, which could be due to non-lethal exposure to rabies or cross-protection induced by exposure to non-rabies bat lyssaviruses that circulate in Cambodia. This observed phenomenon will be further investigated with a cohort of dogs from Stung Treng Province.

Collaborations	Institut Pasteur du Cambodge: Epidemiology and Public Health Unit (Veronique CHEVALIER), and Virology unit (Heidi AUERSWALD)
Funding	Région Occitanie and Swedish Research Council: 2019–2022

Capacity and capability development for *post-mortem* rabies diagnostic at the National Animal Health and Production Research Institute (NAHPRI).

In cooperation with the GIZ funded support to the Royal Government of Cambodia on the improvement of rabies prevention and control (GIZ global programme Pandemic Prevention and Response, One Health), the Virology Unit provides technical expertise on rabies direct diagnostic. With a twinning approach, IPC supports NAHPRI by performing rabies virus diagnosis on animal brain samples via direct fluorescence antibody test (DFAT). The necessary equipment, material, and reagents were assessed early in 2022. The training of four NAHPRI staff members (Pathology and Virology Laboratory) was carried out in 12 training sessions at IPC. During the project, confirmatory testing and sample sharing on request will ensure the quality of testing at NAHPRI.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (Heidi AUERSWALD and Rabies team) Ministry of Agriculture, Forestry and Fisheries (MAFF): National Animal Health and Production Research Institute (NAHPRI, Sothyra TUM) German international cooperation (GIZ)
Funding	German International Cooperation (GIZ): November 2021 – March 2024

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

The Virology Unit's routine rabies diagnostic activities will be documented from 2022 on in a FAO-supported veterinary public health LIMS called SILAB. All samples received for direct diagnostic by DFAT or rabies RT-PCR, and for serological diagnostic by FAVNT offered as a paid service, will be supervised with this LIMS, allowing faster result reporting to customers and to the authorities. After an evaluation of the laboratory's needs regarding rabies diagnostic operations, two LIMS administrators were trained to manage rabies-related activities with SILAB along with four technicians trained for sample and result entry. The implementation of the SILAB LIMS will allow improved sample tracing, testing and reporting, as well as a better overall management of the Virology Unit's diagnostic capacity.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (Heidi AUERSWALD and Rabies Team) Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM, Ercole DEL NEGRO)
Funding	Food and Agriculture Organization of the United Nations (FAO): January 2022 – September 2022 (follow-up financing very likely)

4.4.3 Research Programs – Outlook for 2023

Axis 1: Arboviruses

Chikungunya virus (CHIKV): genetic and phenotypic characterization of CHIKV from the 2020 outbreak in Cambodia.

CHIKV was first detected in Cambodia in 1961 and re-emerged in 2011. After a decade, in 2020, CHIKV was detected again in a large outbreak that spread rapidly across the country. Sequencing carried out on the E genes showed that the latest outbreak's strain belongs to the East/Central/South African genotype with the A226V mutation in the E1 protein that is linked to increased infectivity in *Aedes albopictus* mosquitoes. Three additional mutations in E1 were also observed in strains isolated from Cambodian patients. Our hypothesis is that the new mutations observed in the E gene and potentially other mutations throughout the genome might play an important role in the explosive 2020 outbreak. We plan to conduct comparative viral fitness assays of the CHIKV isolates in several mosquito cell lines and human cell lines.

Additionally, we will conduct a structural analysis of the mutations observed in E1 and E2 genes. These mutations might reflect an antigenic drift in comparison to other CHIKV isolates. An *in-vivo* experiment will be conducted to test whether the new mutations observed in CHIKV samples isolated in 2020 provide any phenotypic advantage in the main mosquito vectors. We will conduct vector competence studies using CHIKV isolates (Asian genotype) from 2011 and 2020 and other Asian and African strains in both *A. aegypti* and *A. albopictus*, to estimate the infection, dissemination, and transmission rates.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (DUONG Veasna, YANNETH Oudamdaniel), and Medical and Veterinary Entomology Unit (Sebastien BOYER) University of Texas Medical Branch (Scott WEAVER)
Funding	Internal funding

Investigation of dog-associated diseases in Southeast Asia (DogZooSEA and SEA-dog-SEA).

An investigation of dog-borne diseases in Southeast Asia through a survey will investigate the perceptions and practices of villagers regarding dog ecology and epidemiology, as well as dog distribution and population dynamics. This project includes sample collection in Cambodia, Indonesia and Thailand. The serological investigation will be performed at the IPC's Virology Unit. Paired serum samples of dogs and their owners will be collected within a follow-up survey to determine the prevalence of arboviruses (dengue, Japanese encephalitis, West Nile viruses) with the respective virus-specific neutralization tests. This will also evaluate the potential of dogs as sentinels for arboviruses. The dog samples will also be analyzed for rabies antibodies to investigate non-lethal exposure to canine rabies. To further strengthen the laboratory capacity for zoonotic diseases in the region, IPC organized and hosted a training on arbovirus neutralization methods in September 2022 with participants from One Health Collaborating Center of the Gadjah Mada University (Yogyakarta, Indonesia), from the Faculty of Veterinary Technology of the Kasetsart University (Bangkok, Thailand), and from the *Institut Pasteur du Laos'* Rabies Group (Vientiane, Lao PDR).

Collaborations	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)
Funding	FSPI-One Health in Practice in Southeast Asia (OH SEA): February 2022 – March 2023

Virological investigation of emerging pathogens in Cambodia (VIPCAM).

This pilot project aims to develop a bioinformatics platform with a powerful performance system to ensure the proper analysis of NGS data produced continuously at IPC through various surveillance and research projects.

Collaborations	Institut Pasteur du Cambodge: Virology unit (YANNETH Oudamdaniel, Jurre SIEGERS, Janin NOUHIN, OU Teyputita) and Sequency Mini-Platform (HEANG Vireak) Mahidol University, Thailand (Prof. Thanat CHOOKAJORN),
Funding	Mahidol University, Thailand: 2022–2023

Virulence evolution of the Japanese encephalitis virus (JEV) along the swine–mosquito–human axis in Asia.

The proposal aims to decipher JEV virulence evolution features in Vietnam and Cambodia. How JEV evolves along the host axis involving pigs, mosquitoes, and humans; and how changes in the viral genome are linked to its virulence's evolution, are the key scientific questions for deciphering the epidemiological trends of JE. Answering these questions will provide valuable knowledge for updating the JEvaccine program, reducing the disease burden in the healthcare system, and alleviate poverty in Vietnam and Cambodia. Within this project, sentinel swine as well as mosquitoes caught repeatedly in close proximity will be analysed for JEV presence, and virus isolation attempts will be performed on positive samples. Furthermore, a comparative analysis of the *in-vitro* evolution of Cambodian JEV isolates from various species (swine, mosquitoes, humans) will be performed by serial, alternating passaging in swine and mosquito cells.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (Erik KARLSSON, YANNETH Oudamdaniel, Heidi AUERSWALD); Uppsala University, Sweden (Jiaxin LING, Johanna LINDAHL)
Funding	Swedish Research Council: 2023–2026

Deterioration of acute dengue virus (DENV) infection.

The project aims to obtain clinically characterized blood samples (serum, EDTA-plasma) from patients presenting with acute febrile illness in whom acute Dengue infection is confirmed. These will be further analyzed for biomarkers to discriminate early between DENV infections with mild and severe clinical outcome.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (Erik KARLSSON, DUONG Veasna, and YANNETH Oudamdaniel); Epidemiology and Public Health Unit (LY Sowath, SORN Sopheak); Roche Diagnostics GmbH, Germany (Maren REINERS)
Funding	Roche Diagnostics GmbH: 2023–2024

Understanding the molecular determinants of emerging chikungunya virus (CHIKV) variants to harness heterologous immunity to CHIKV and related alphaviruses for enhanced diagnostics, therapies and vaccines.

The overarching goal of this PIU is to establish a functional and integrated research platform for the systematic functional characterization of emerging CHIKV variants and related arthritogenic alphaviruses, including the viruses' capacity to escape protective immunity. The findings will help inform the development of novel diagnostic tools, therapeutic treatment options and prophylactic vaccines. This will allow us to generate predictions and development treatment strategies against new CHIKV variants and related alphaviruses to better prepare for future infection outbreaks.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (DUONG Veasna, Heidi AUERSWALD and YANNETH Oudamdaniel) McGill University, McGill Research Center on Complex Traits (Jörg Hermann FRITZ and Silvia VIDAL)
Funding	Submitted to PIU for funding. 2023–2027

Obtaining accurate estimates of disease burdens from biased surveillance data.

In this project, we will answer the following question: can spatially and temporally-biased case and genome data be used to provide an accurate description of pathogen spread and burden throughout a country?

This collaboration between the University of Cambridge, the University of Southampton and Institut Pasteur du Cambodge (IPC) will maximise our ability to identify the population and environmental features that drive DENV spread in Cambodia, and to estimate the underlying infection burden across the country. We will use DENV sequences and monthly case data from around the country. In addition, we will work with researchers at the University of Southampton who have amassed detailed national, regional, and global datasets that capture the changing human and environmental geography of countries around the world, including Cambodia (www.worldpop.org).

The project is divided into two specific aims:

- Specific aim 1: Estimate the DENV infection burden across Cambodia from 2010 to 2023 using catalytic models applied to results of the age distribution of cases in each district.
- Specific aim 2: Develop a suite of models that can reconstruct the burden of DENV using biased genome and case data from 2010 to 2023, and compare the performance of these models to the burden estimated in Aim 1.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (DUONG Veasna); Epidemiology and Public Health Unit (Claude FLAMAND); University of Cambridge, Department of Genetics (Henrik SALJE) University of Southampton
Funding	Submitted to Wellcome Trust

Axis 2: Respiratory Viruses

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.

Collaborations	Institut Pasteur du Cambodge: Virology unit (Erik KARLSSON and Heidi AUERSWALD), St. Jude Children's Research Hospital (Stacey SCHULTZ-CHERRY)
Funding	CEIRR/NIAID; APPROVED – Funding Discussion Initiated March 2023 (2023–2025)

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

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

Collaborations	Institut Pasteur du Cambodge: Virology Unit (Erik KARLSSON) Cambridge (Jim KAUFMAN) Ben-Gurion University of the Negev (Tomer HERTZ)
Funding	Wellcome Trust; PENDING – To be submitted July 2023

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.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (Erik KARLSSON, Heidi AUERSWALD and YANNETH Oudamdaniel) Theramo
Funding	FAO; Started March 2022 and continues in 2023

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.

Collaborations	Institut Pasteur du Cambodge: Virology unit (Erik KARLSSON) Duke-NUS (Gavin SMITH)
Funds	Bill and Melinda Gates Foundation; Started November 2022

Axis 3: Zoonoses

HANTA-FRAKAM Project: Improving our Knowledge of Hantavirus Circulation in France and Cambodia.

The proposal aims to 1) improve serological and molecular assays for detection and characterization of hantavirus, 2) conduct molecular epidemiology analysis of hantavirus infection in rodents and humans to better understand their circulation in France and Cambodia, 3) assess disease burden and risk factors associated with hantavirus infection in humans in Cambodia, and 4) study the Thailand hantavirus' natural history of infection in rodent models.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (J. NOUHIN, V. HUL, J. GUILLEBAUD, S. KEN, and V. DUONG) and Epidemiology and Public Health Unit (S. LY, S. SORN, and K. NGUON) Institut Pasteur Paris (J.-M. REYNES, J. VANHOMWEGEN, V. CARO, and V. SAUVAGE)
Funds	PENDING - To be submitted to funding agencies (i.e.: ANRS|MIE)

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

We aim to identify tick species and associated pathogens including virus and bacteria circulating in Cambodia. Additionally, we aim 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.

Research activities was delayed and limited in 2022 (purchasing reagents and commercial ELISA kits).

Collaborations	Institut Pasteur du Cambodge: Virology Unit (J. NOUHIN, L. KHUN, L. HENG, and V. DUONG); Medical and Veterinary Entomology Unit (P.-O. MARQUART, S. YEAN, K. HENG, S. LENG, and S. BOYER); Epidemiology and Public Health Unit (S. SORN and S. LY) Institut Pasteur Paris (S. BONNET, R. PAUL, M. ELOIT, and A. SAKUNTABHAI)
Funding	NIH (Award Number 3U01AI151758-03S1); August 2022 – May 2023

Biodiversity Conservation to mitigate the risks of emergING infectious diseases (BCOMING).

The project's aim is to co-develop innovations with all stakeholders in biodiversity hotspots to reduce the risk of infectious disease emergence through biodiversity conservation, restoration and surveillance.

The kick-off meeting was held in France in September 2022, and field activities are planned to start in March 2023.

Collaborations	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)
Funding	EU (HORIZON-CL6-2021-BIODIV-01-11); 2022–2026

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.

Collaborations	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)
Funding	French Development Agency (AFD); 2022–2024

Expanding early warning surveillance using novel collection and sequencing techniques.

The objective of this proposal is to expand air-based surveillance using novel drone technologies and cutting-edge detection and sequencing methods and to benchmark their performance against standard surveillance techniques.

Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik KARLSSON)
Funding	FAO Innovation Award; 2023 PENDING signing Letter of Agreement

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

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 Rhinophilid species adept at carrying SARS-CoV-2-related viruses and the potential impacts of reverse zoonoses.

Collaborations	Institut Pasteur du Cambodge: Virology Unit (Erik KARLSSON) Cambridge University (Jim KAUFMAN) Duke-NUS (Gavin SMITH)
Funding	Wellcome Trust; 2022–2025 PENDING – to be submitted July 2023

Axis 4: Rabies and Other Viruses

Investigation of dog-associated diseases in South-East Asia (DogZooSEA and SEA-dog-SEA).

As previous IPC dog studies have shown the presence of rabies nAb in dogs without vaccination history, we aim to analyze dogs from rural communities in Stung Treng province via FAVNT. As these communities have reportedly no access to dog rabies vaccination, we will gain further insights into the prevalence of rabies nAb that are not induced by rabies vaccination. These antibodies could have been induced due to low-dose/non-lethal exposure to rabies or exposure to non-rabies bat lyssaviruses that circulate in Cambodia.

Collaborations	Institut Pasteur du Cambodge: Epidemiology and Public Health Unit (LY Sowath and Helene GUIS), and Virology Unit (Heidi AUERSWALD)
Funding	Solidarity Fund for Innovative Projects (FSPI): February 2022 – March 2023

Serological long-term follow-up of patients who received the IPC's PEP regimen (RESIST-3 project). The Rabies Elimination Support through Integrative Science and salvage Therapy-3 (RESIST-3) study is an integrated Pasteur research programme aiming to help identify tools to support the fight against rabies. In detail, it aims to investigate the development and persistence of rabies nAb in people that received the IPC PEP regimen. Therefore, serum samples from PEP recipients will be analysed via FAVNT before vaccination (D0), shortly after vaccination (D7 and D14), and six months and one year after PEP treatment.

Collaborations	Institut Pasteur du Cambodge: Epidemiology and Public Health unit (LY Sowath), Immunology unit (Tineke CANTAERT) and Virology Unit (Heidi AUERSWALD)
Funding	ACIP and Bourse de la Fondation Anne Maurer-Cecchini: January 2021 – December 2022

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

The main objective of our study is to describe and characterize HPV diversity circulating in HIV-infected women in Cambodia using the NGS approach. Secondarily, we will assess the associations between variants in HPV E6 and E7 coding genes and CIN status.
The expected outcomes are 1) the acquisition of HPV full-length genome sequencing competency, 2) a description of HPV diversity in HIV-infected Cambodian women, and 3) clinically significant data regarding the relationships between variations of HPV E6 and E7 coding regions and the occurrence of CIN.

Due to COVID-19, research activities were delayed. Sequencing has started in 2022 in 20 among about 60 samples selected for the study.

Collaborations	Institut Pasteur du Cambodge*:* Virology Unit (J. NOUHIN, N. BOUKLI, J. GUILLEBAUD, and L. KHUN) and Sequencing Platform (N. KHIM and V. HEANG) Calmette Hospital (S. LIMSRENG, A. KORN) University of Health Sciences (S. KIM, S. MOEUNG) ANRS (O. SEGERAL) IRD (P. De BEAUDRAP)
Funding	IPC Internal funding; 2022–2023

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

In this study, we aim to 1) develop a phenotyping assay to study DAA resistance of HCV genotype 6 that has been less studied unlike genotypes predominant in western countries, and 2) to implement this phenotypic assay to evaluate the clinical significance of the substitutions found in our previous study. The expected outcomes are an ability to acquire a phenotypic assay available to investigate HCV DAA resistance in Cambodia. In addition, we will be able to determine the clinical significance of newly emerged substitutions in NS5A and NS5B regions.

The research activities were delayed in 2022. Due to COVID-19 situation in China, the shipment of plasmid and cell lines necessary for the experiment from IP Shanghai was postponed until 2023

Collaborations	Institut Pasteur du Cambodge: Virology Unit (J. NOUHIN, H. AUERSWALD, and V. DUONG) Institut Pasteur Shanghai (J. Zhong)
Funding	IPC Internal funding; 2022–2023

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 (LBM). 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 17). DENV-3 was the main serotype during the major outbreak in 2007, followed by DENV-2 in subsequent years until 2010. DENV-1 became the main serotype detected from 2011 to 2015, and 2016 was marked by an increased detection of DENV-2. The Virology Unit continued to detect mainly DENV-2 in 2017–2018, while DENV-1 was still present in the country at a lower level. The year 2019 was marked by another major dengue outbreak caused by

DENV-1 with more than 68,000 hospitalized cases and 48 deaths reported throughout the country. Since 2020, we have observed a decrease in the number of reported cases, particularly in 2021 (only 1903 reported cases). In 2022, DENV-2 is still the dominant serotype, followed by DENV-4 and DENV-1 (2 cases). DENV-3 was not detected for 2 years consecutively since 2021.

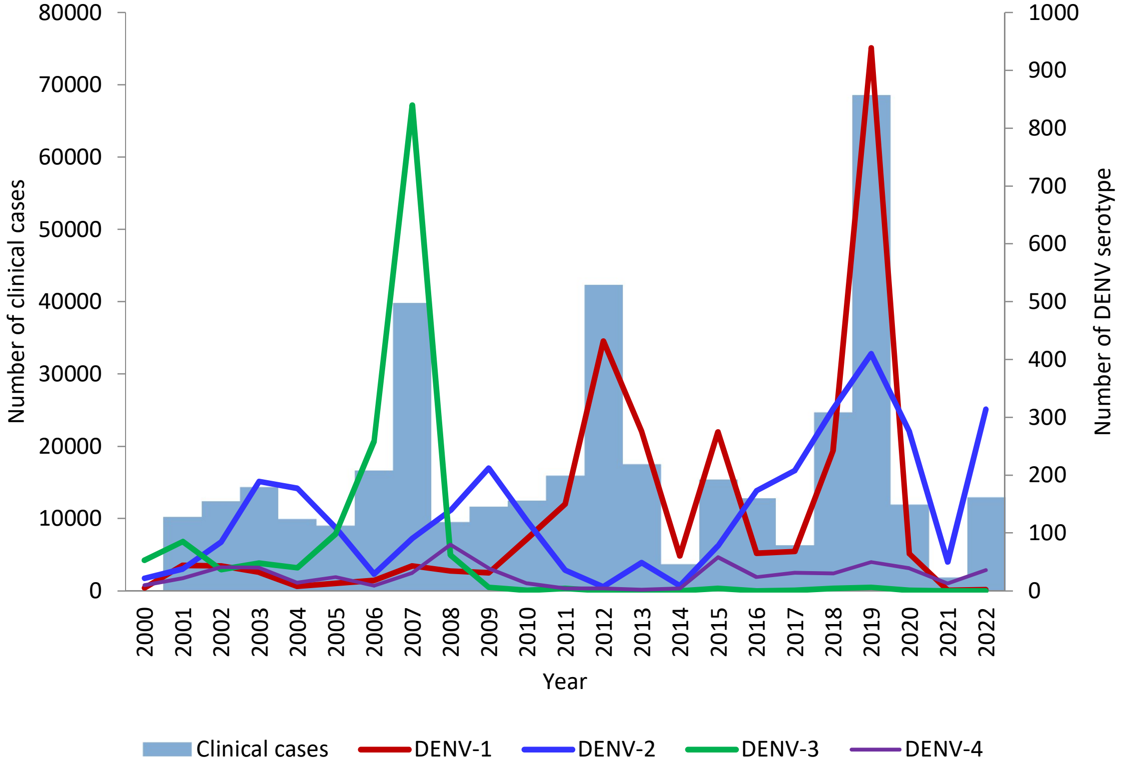


Figure 17. Dengue virus serotypes circulation in Cambodia and number of dengue clinical cases reported.

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

IPC's Virology Unit has been Cambodia's National Influenza Center (NIC) since 2006. The Virology Unit at IPC was designated as a WHO H5 Reference Laboratory of the WHO Global Influenza Surveillance and Response System (GISRS) in October 2014. In April 2020, the work done at IPC in response to the global COVID outbreak was recognized by naming the Virology Unit as a WHO COVID-19 Global Referral Laboratory. Our activities carried out in support of the Cambodian national authorities and of our international partners, as described below for 2022, will continue in 2023 and beyond.

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

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

Human seasonal influenza.

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

The current global COVID-19 pandemic has significantly altered both the surveillance and landscape of respiratory 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 (Figure 18).

Figure 18. Number of influenza cases detected in sentinel and outbreak samples 2020-2022. 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 2022 and sequencing, analysis, and publication are underway to describe these viruses in Cambodian children expected mid- to late 2023.

Assessment of Multiplex Testing for RSV, Influenza, and SARS-CoV-2.

Molecular multiplex assays (MPAs) for the simultaneous detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza and respiratory syncytial virus (RSV) in a single RT-PCR reaction reduce time requirements and increase efficiency for identifying multiple pathogens with overlapping clinical presentation but different treatments or public health implications. The clinical performances of XpertXpress® SARS-CoV-2/Flu/RSV (Cepheid, GX), TaqPathTM COVID-19, FluA/B, RSV Combo kit (Thermo Fisher Scientific, TP), and PowerChekTM SARS-CoV-2/Influenza A&B/RSV Multiplex RT-PCR kit II (KogeneBiotech, PC) were compared to individual Standards of Care (SoC). Thirteen isolates of SARS-CoV-2, human seasonal influenza, and avian influenza served to assess the limit of detection (LoD). Then, positive and negative residual nasopharyngeal specimens, collected under public health surveillance and pandemic response served for evaluation. Subsequently, a comparison of effectiveness was carried out. The three MPAs confidently detect all lineages of SARS-CoV-2 and influenza viruses. MPA-LoDs vary by 1-2 Log10 differences from SoC depending on assay type and virus strain. Clinical evaluation resulted in an overall agreement between 97 % and 100 %, demonstrating a high accuracy to detect all targets. Existing differences in costs, testing burden and implementation constraints influence the choice in primary or community settings. TP, PC and GX reliably detect SARS-CoV-2, influenza and RSV simultaneously, with reduced time-to-results and simplified workflows. MPAs have the potential to enhance diagnostics, surveillance system, and epidemic response to drive policy on prevention and control of viral respiratory infections.

Novel coronavirus disease 2019 (COVID-19).

Following the detection of a cluster of cases of pneumonia of unknown etiology in Wuhan, China in December, 2019, IPC began to immediately organize screening processes for individuals should any cases be detected in Cambodia. Through IPC's previous involvement in the Pandemic Preparedness for Global Health (PREDICT) program, IPC was quickly able to mobilize and prepare to begin testing suspected COVID-19 samples in early January 2020. In addition, IPC quickly obtained and established protocols for the real-time molecular diagnosis of SARS-CoV-2 through WHO-provided protocols. As such, using our BLS-3–level capacity in conjunction with multiple diagnostic tests, IPC ensured diagnostic capacity in Cambodia very early and was approved as a first-line laboratory for diagnosis of COVID-19 (SARS-CoV-2) on 21 January 2020. 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. In addition, the epidemiology and public health unit at IPC is using its extensive experience to facilitate data management, reporting, and contact tracing. While testing has significantly decreased in 2022, **by 31 December 2021, IPC had tested over 942,703 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. While sequencing remains the "gold standard" for SARS-CoV-2 variant detection, with the global spread of COVID-19 variants of concern (VoC), numerous commercial kits for the molecular detection of VoC lineages of SARS-CoV-2 have become available on the market, touting quick, sensitive, and specific detection of various VoCs in suspected cases. In 2021, IPC validated five of these kits, and this data is frequently shared with the global network. In addition, technology transfer has been achieved with NIPH, Sihanoukville, and Ket Mealea laboratories as beneficiaries to help ensure that testing for all VoCs is widely available in the country. A publication detailing this work is expected by mid-2023.

Based on the validation/verification and usability of the VoC RT-PCR kits tested, IPC decided to employ the KogeneBiotech PowerChek™ SARS-CoV-2 S-gene mutation kit in daily routine genomic surveillance. IPC aims to test 92 samples/day (644/week) in routine testing of new cases in Cambodia. These samples are chosen based on 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 2022, IPC had tested over 20,933 samples for VoC by RT-PCR to monitor Variants of Concern (VoC) circulating in and entering Cambodia (Figure 19).

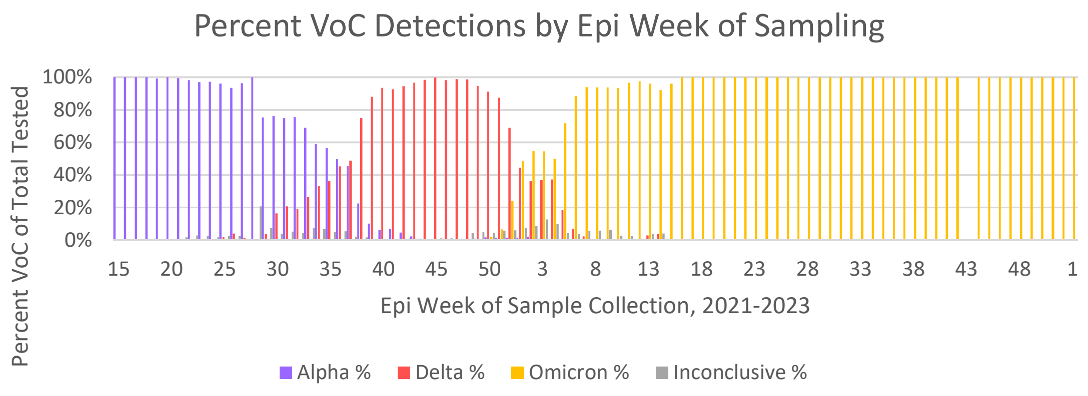


Figure 19. Detection of VOC in community samples in Cambodia by epidemiological week from April, 2021. Due to declining sample numbers after week 50 of 2021, percentages may not reflect true prevalence in the community.

SARS-CoV-2 Sequencing.

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 2022, IPC was able to sequence 3,357 samples (2.42 % of the total reported cases at that time) and submitted results to GISAID (Figure 20). Further work continues to monitor mutation rates in vaccinated versus unvaccinated individuals, transmission dynamics, and the phylogeography of the viral spread. Two manuscripts were drafted regarding SARS-CoV-2 sequencing in Cambodia.

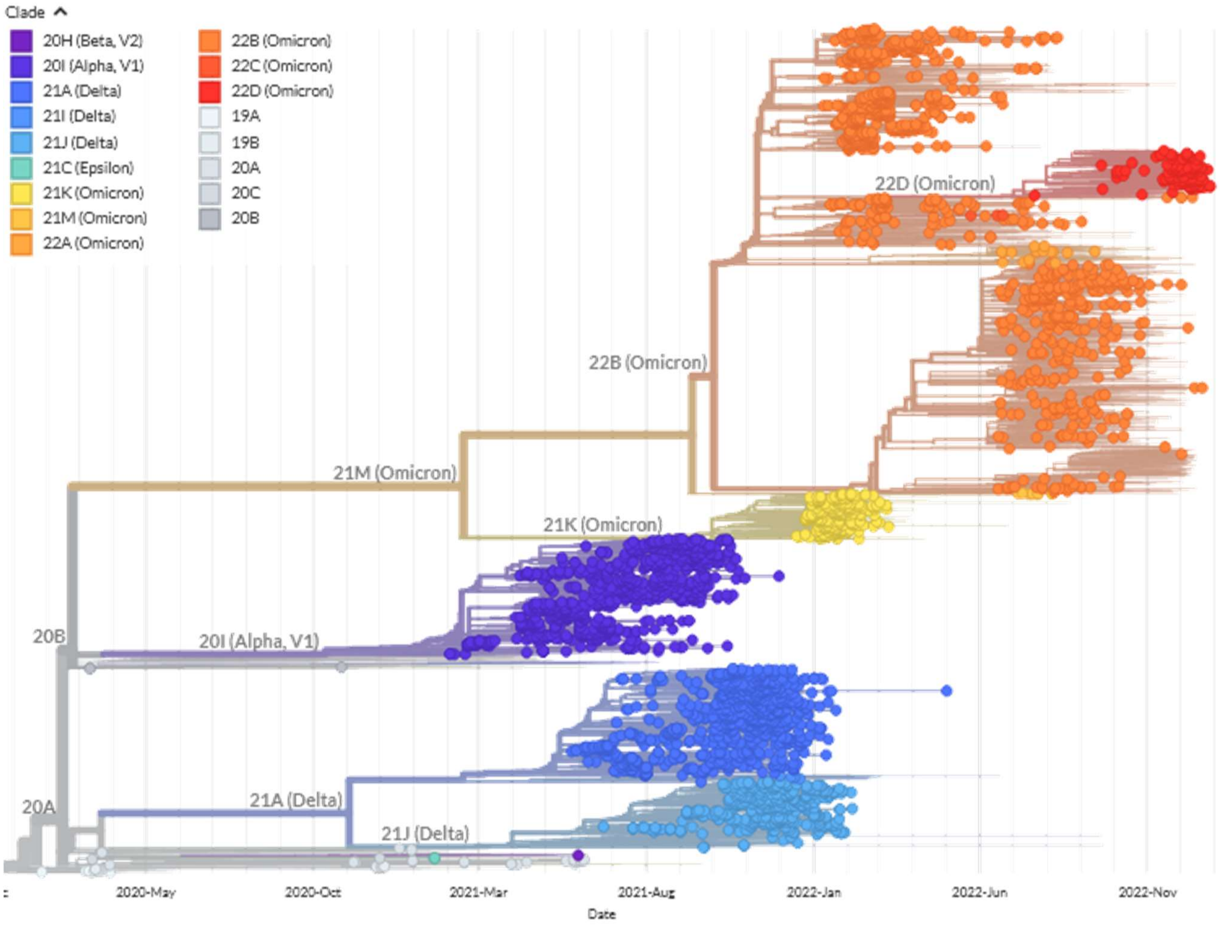


Figure 20. Timescale phylogeny of SARS-CoV-2 sequences (n=3,851) between Jan 27th 2020 and end of 2022. Cambodian sequences are highlighted. Sequences from both IPC and NIPH are included in analysis as available from GISAID.

Monitoring Omicron and other VoC.

VoCs are identified based on sequencing data and outputs from PANGOLIN (Figure 21), Omicron subvariants groups BA.1, BA.2, BA.4, and BA.5 were detected in Cambodia in 2022. 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 at week 23, mostly with BA.5.2. BA.2.75 was first detected in one in week 29 and became the dominant variant at week 44 of 2022. Other subvariants detected include BE, BF, BL, BM, BN, BQ, BW, CH, CP, XAZ, and XBB, and increased in prevalence since week 44, 2022. Subvariant BN (BN.1.2, BN.1.3, BN.1.4, BN.1.5) was dominant among "other" detections after week 50 in 2022. However, BA.2.75 still represents the majority of sequenced strains. One sample of BQ.1.1 was detected in week 48, 2022.

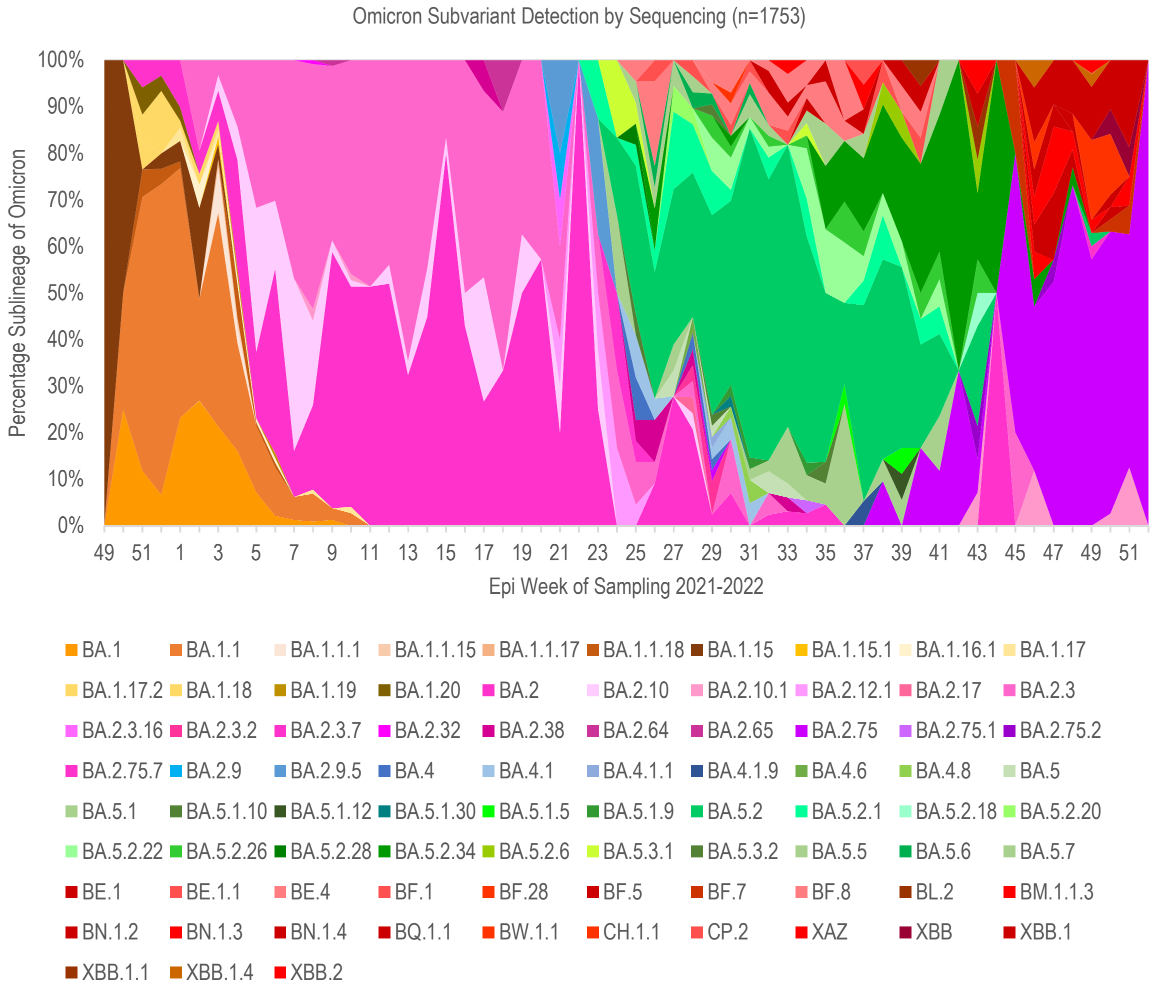


Figure 21. Detetction of lineages of subvaraints of Omicron in fully sequenced samples (n=1753) by epidemiological week of sampling between 14 Dec, 2021 and the end of 2022. The percentage for each subvariant is represented as part of the entire sequencing for that individual week. BA.1 is represented in orange, BA.2 in pink, BA.4 in blue, BA.5 in green colors. "Other" newly emerging subvariants such as BE, BF, BL. BM, BN, BQ, BW, CH, CP, XAZ, and XBB are in red colors. Sequences from both IPC and NIPH are included in the analysis, as available through 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 22). 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. Genomic epidemiology for Alpha and Delta waves in 2021 has been completed, and publication is expected in mid-2023.

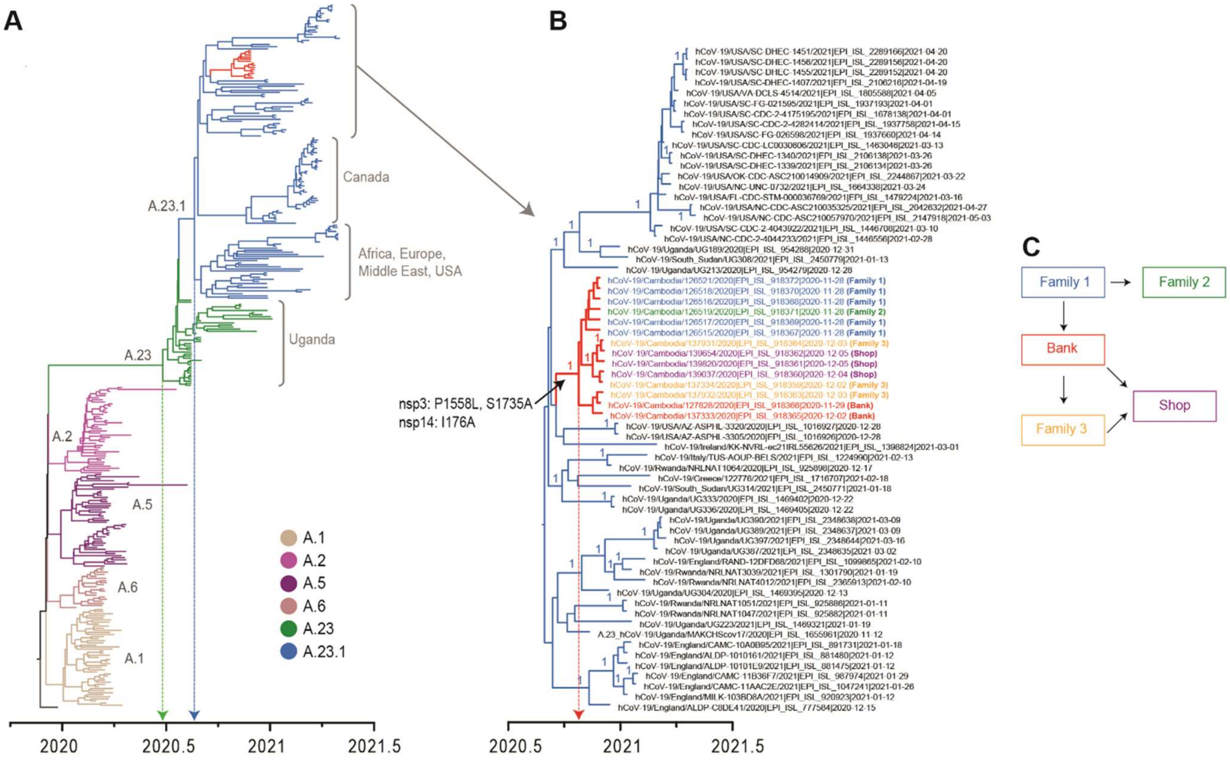


Figure 22. Temporal phylogeny of A.23 variants and related SARS-CoV-2 lineage A sequences (n=339). ***(A)*** *The maximum clade credibility (MCC) tree was reconstructed based on the whole genome of SARS-CoV-2 from Cambodia and globally. Colored branches represent various lineage A viruses. The green 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.*

Viral Isolation and Titration.

Having a BSL-3 level facility and experience in isolating numerous types of viruses, IPC was quickly able establish viral isolation and tittering (both TCID50 and PFU) for COVID-19 in Cambodia. At the current time, 129 viral isolates (Wuhan-like, Alpha, and Omicron) are available from patients identified in Cambodia. We have as yet been unsuccessful in isolating 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.

Rabies remains a major public health concern in Cambodia. IPC's Virology Unit has been involved in the diagnosis of rabies infections post-mortem in animal brains using a DFAT for almost two decades. This test is routinely performed on samples obtained from suspected rabies-infected animals, usually provided by patients seeking medical treatment in one of IPC's PEP centres. Between 2000 and 2021, the average number of animals brought yearly to IPC for rabies diagnosis was 201, with a positivity rate at 54.2 %. In 2022, the Virology Unit received 239 animal brain samples, and confirmed rabies infection in 73.6 % of the samples (Figure 23). Furthermore, the DFAT capability of IPC was increased by expanding the rabies team to four technicians who can perform this test routinely.

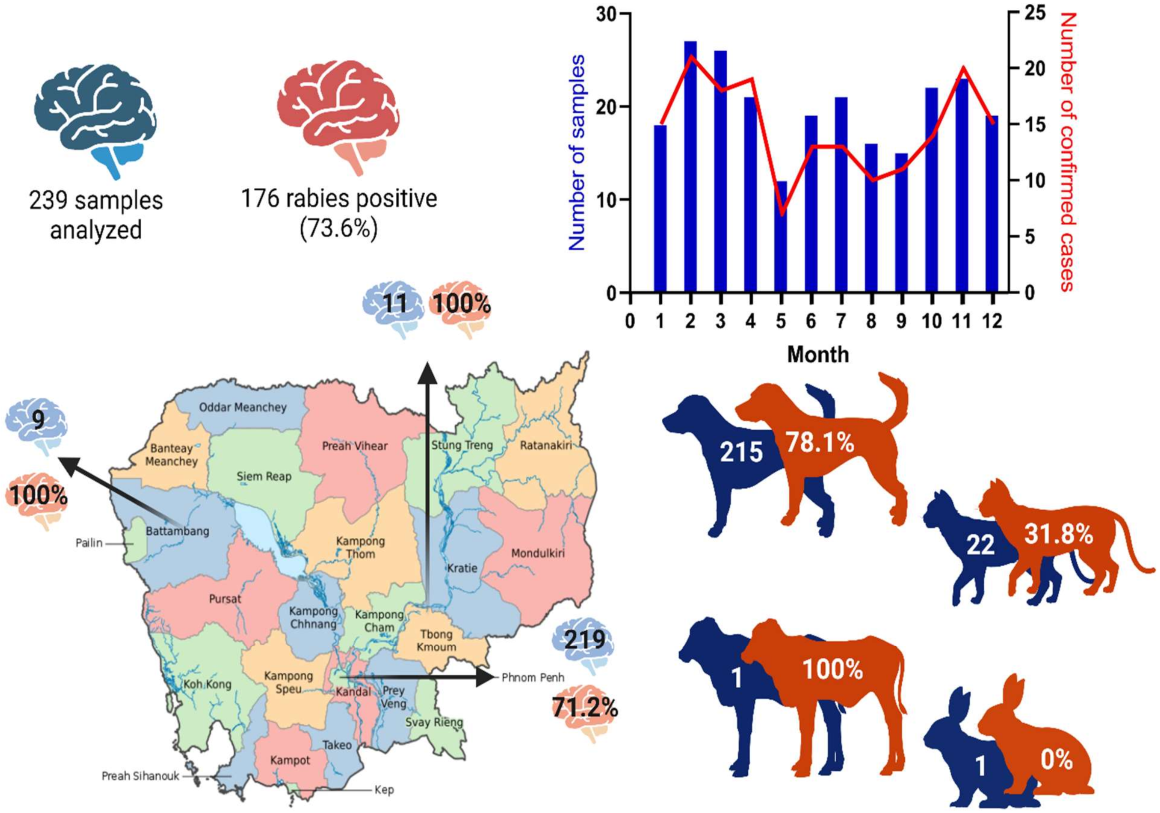


Figure 23. Summary of rabies surveillance in 2022 from animal samples received at Institut Pasteur du Cambodge

Development of a massive open online course (MOOC) and other rabies information, education and communication (IEC) material.

In cooperation with the GIZ-funded support to the Royal Government of Cambodia on the improvement of rabies prevention and control (GIZ's global Pandemic Prevention and Response programme, One Health), the Virology Unit provides scientific expertise in the development of a

MOOC, aiming to increase the knowledge of teachers on the topics of rabies and One Health. This MOOC will be implemented by the School Health Department (SHD) of the Ministry of Education, Youth and Sports (MoEYS). Furthermore, IPC shared its own rabies IEC material with the Ministry of Health (MoH), MAFF and MoEYS (through GIZ) to help developing updated and harmonized IEC materials.

Support of the National Strategic Plan (NSP) for Animal Health Laboratories for 2023–2030.
IPC, and especially the Virology Unit, is a partner in the Cambodian network of animal health laboratories. Therefore, IPC supported the PVS (Performance of Veterinary Services) evaluation of NAHPRI by WOAH in 2022. Furthermore, IPC's Virology Unit will continue aiding the development of NAHPRI's NSP for 2023–2030, and the improvement of intersectoral collaboration between animal health laboratories in Cambodia.

4.4.5 Teaching and Training

One of IPC's main missions is to provide teaching and training opportunities. The Virology Unit has been proactive in the training of students and staff from national and international institutions, as well as government partners in the fields of surveillance and research.

Teaching and Training Activities

- Scientists from the Virology Unit are actively involved in teaching bachelor and master programs at University of Health Science, Cambodia (UHS,) including the Master's Degree in Infectiology and the Master's Degree in Medical Biology.
- Training in rabies diagnostic for staff from governmental partners like NAHPRI will continue upon request.
- Dr. Heidi Auerswald and the Virology Unit's serology team organized an international course on the diagnosis of arboviruses at the *Institut Pasteur du Cambodge* as part of the DogZooSEA project, on 19–23 September 2022, for six scientists: two coming from the One Health Collaborating Center of the Gadjah Mada University (Yogyakarta, Indonesia), two from the Faculty of Veterinary Technology of the Kasetsart University (Bangkok, Thailand) and two from the Rabies Group at *Institut Pasteur du Laos* (Vientiane, Lao PDR).

Training Received

- Mrs Janin Nouhin attended a bioinformatics workshop organized by *l'Institut de Recherche en Santé, de Surveillance Epidémiologique et de Formation* (IRESSEF) and by CIGASS-UCAD. Dakar, Senegal. 10–15 October 2022.
- Four staff from the Virology Unit attended The Disease Ecology Symposium and Workshop Training 2022: "The Ecology of Wildlife-Borne Diseases in a Post-COVID Era" at Mahidol University, Kanchanaburi, Thailand, from 28 November to 2 December 2022.
- Ms Ou Teyputita attended 1) a virtual training entitled "Virus Genomics and Evolution 2022" given at the Wellcome Genome Campus, Hinxton, Cambridge, 9 to 11 November 2022; 2) an onsite training on "Genomics and Clinical Virology", organised par the Wellcome Genome Campus, Hinxton, Cambridge, 26 June – 1 July 2022; and 3) a virtual training on "Next Generation Sequencing Bioinformatics" by Wellcome Genome Campus, Hinxton, Cambridge, 17-21 January 2022.

- Dr Heidi Auerswald**,** Chea KimLay, Hurm Srey Viseth, Heng Leangyi and Yanneth Oudamdaniel attended an in-person SILAB LIMS training provided by the *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise* (IZSAM) with support from the FAO at IPC, 4-15 July 2022
- Dr Heidi Auerswald attended 1) an on-site demonstration of SILAB LIMS at *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise* (IZSAM) in Teramo, Italy, 6 June 2022 and 2) an online training on effective training design and management provided by he Academy for International Cooperation Asia (AIZ Bangkok) and financed by GIZ. 17-22 June 2022.
- Mrs Rath Sophoannadedh and Mr Sin Sarath attended a two-week training on sequencing and virus isolation at WHO CC Influenza Melbourne, Australia, in October
- Several staff at the Virology Unit received language training provided by IPC.

PhD students

- Mr Hul Vibol is in the final year of his PhD program (2019–2023 with a 6-month extension) at the Aix-Marseille University, working on the characterization of two novel arenaviruses and their seroprevalence in Cambodia.
- Ms Ou Teyputita is in the second year of her PhD program at the University of Montpellier, France, and is expected to complete the degree by 2024. She studies the coronaviruses and SARS-CoV-related viruses circulating in bats in Cambodia.

Master's Students

- Mr Jimmy Cadenes, first-year master's student from *Université Paris Saclay*, spent twelve weeks with IPC from September to December 2022, working on the detection and characterization of infectious bronchitis virus (IBV) in Cambodian poultry.
- Mr David Norte, first-year master's student from *Université Paris Saclay, spent* twelve weeks with IPC from May to July 2022 working on the detection and characterization of paramyxovirus and rhabdovirus among bats samples in Cambodia.
- Mrs Yann Sokhoun, a second-year student in the Master's Program on Infectious Diseases at UHS, spent twelve weeks between January and April 2022 working on the characterization of influenza virus in poultry from live bird markets.
- Ms Hoem Thavry, a second-year student in the Master's of Science in Epidemiology program at the National Institute of Public Health, Cambodia.

Bachelor Students

- Mr Heng Leangyi, first-year bachelor's student in Medical Laboratory Technology at the University of Health Sciences, Cambodia
- Mrs. In Sophea, first-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

4.4.6 Outlook

Since 2021, the Virology Unit expanded has exponentially. Increases occurred not only in size, but also in number of activities, covering a wide range of research areas in the laboratory and in the field. In 2023, the Unit is being restructured to delegate responsibilities to senior scientists and talented staff, and to meet the Unit's new research objectives. 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 publish the huge amount of data generated, which has been significantly delayed by the urgent response to the COVID-19 pandemic.

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;
- A bioinformatician will be recruited to strengthen bioinformatics analysis expertise in the Unit;
- 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;
- 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;
- 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 Publications List in 2022

NOTE

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

1. **Rapid Generation of In-House Serological Assays Is Comparable to Commercial Kits Critical for Early Response to Pandemics: A Case With SARS-CoV-2.**
   Heidi Auerswald, Chanreaksmey Eng, Sokchea Lay, Saraden In, Sokchea Eng, Hoa Thi My Vo, Charya Sith, Sokleaph Cheng, Gauthier Delvallez, Vann Mich, Ngy Meng, Ly Sovann, Kraing Sidonn, Jessica Vanhomwegen, Tineke Cantaert, Philippe Dussart, Veasna Duong, Erik A. Karlsson.
   Front Med. 2022;9:864972.
2. **A Look inside the Replication Dynamics of SARS-CoV-2 in Blyth's Horseshoe Bat (Rhinolophus lepidus) Kidney Cells."**
   Heidi Auerswald, Dolyce H. W. Low, Jurre Y. Siegers, Teyputita Ou, Sonita Kol, Saraden In, Martin Linster, Yvonne C. F. Su, Ian H. Mendenhall, Veasna Duong, Gavin J. D. Smith, Erik A. Karlsson.
   Microbiology Spectrum. American Society for Microbiology 0(0):e00449-22.

3. **Accessibility to rabies centers and human rabies post-exposure prophylaxis rates in Cambodia: A Bayesian spatio-temporal analysis to identify optimal locations for future centers**
   Jerome N. Baron, Véronique Chevalier, Sowath Ly, Veasna Duong, Philippe Dussart, Didier Fontenille, Yik Sing Peng, Beatriz Martínez-López.
   PLoS Negl Trop Dis. 2022;16(6):e0010494.

4. **Dengue virus NS1 protein conveys pro-inflammatory signals by docking onto high-density lipoproteins**
   Souheyla Benfrid, Kyu-Ho Park, Mariano Dellarole, James E Voss, Carole Tamietti, Gérard Pehau-Arnaudet, Bertrand Raynal, Sébastien Brûlé, Patrick England, Xiaokang Zhang, Anastassia Mikhailova, Milena Hasan, Marie-No€elle Ungeheuer, Stéphane Petres, Scott B Biering, Eva Harris, Anavaj Sakuntabhai, Philippe Buchy, Veasna Duong, Philippe Dussart, Fasséli Coulibaly, François Bontems, Felix A Rey, Marie Flamand.
   EMBO reports. John Wiley & Sons, Ltd; 2022;n/a(n/a):e53600.

5. **Evaluation of Zika rapid tests as aids for clinical diagnosis and epidemic preparedness**
   Debi Boeras, Cheikh Tidiane Diagne, Jose L. Pelegrino, Marc Grandadam, Veasna Duong, Philippe Dussart, Paul Brey, Didye Ruiz, Marisa Adati, Annelies Wilder-Smith, Andrew K. Falconar, Claudia M. Romero, Maria Guzman, Nagwa Hasanin, Amadou Sall, Rosanna W. Peeling.
   eClinicalMedicine. 2022;49:101478.

6. **Early Changes in Interferon Gene Expression and Antibody Responses Following Influenza Vaccination in Pregnant Women.**
   Raquel Giacomelli Cao, Lisa Christian, Zhaohui Xu, Lisa Jaramillo, Bennett Smith, Erik A Karlsson, Stacey Schultz Cherry, Asuncion Mejias, Octavio Ramilo.
   The Journal of Infectious Diseases. 2022;225(2):341-51.

7. **Pestilence and famine: Continuing down the vicious cycle with COVID-19**
   Sudipta Hyder, Rethy K. Chhem, Filip Claes, Erik Albert Karlsson.
   PLOS Pathogens. Public Library of Science; 2022;18(10):e1010810.

8. Living Safely With Bats: Lessons in Developing and Sharing a Global One Health Educational Resource

Martinez, S., A. Sullivan, E. Hagan, J. Goley, J. H. Epstein, K. J. Olival, K. Saylors, J. Euren, J. Bangura, S. Zikankuba, M. M. M. Mouiche, A. O. Camara, J. Desmond, A. Islam, T. Hughes, S. Wacharplusadee, V. Duong, N. T. T. Nga, B. Bird, T. Goldstein, D. Wolking, C. K. Johnson, J. A. Mazet, S. H. Olson, A. E. Fine, M. Valitutto, W. B. Karesh, P. Daszak, L. Francisco and PREDICT Consortium (2022).
Glob Health Sci Pract 10(6).

9. Zika vector competence data reveals risks of outbreaks: the contribution of the European ZIKAlliance project

Thomas Obadia, Gladys Gutierrez-Bugallo, Veasna Duong, Ana I. Nuñez, Rosilainy S. Fernandes, Basile Kamgang, Liza Hery, Yann Gomard, Sandra R. Abbo, Davy Jiolle, Uros Glavinic, Myrielle Dupont-Rouzeyrol, Célestine M. Atyame, Nicolas Pocquet, Sébastien Boyer, Catherine Dauga, Marie Vazeille, André Yébakima, Michael T. White, Constantianus J. M. Koenraadt, Patrick Mavingui, Anubis Vega-Rua, Eva Veronesi, Gorben P. Pijlman, Christophe Paupy, Núria Busquets, Ricardo Lourenço-de-Oliveira, Xavier De Lamballerie, Anna-Bella Failloux. Nat Commun.
Nature Publishing Group; 2022;13(1):4490.

10. Predicting the potential for zoonotic transmission and host associations for novel viruses

P. S. Pandit, S. J. Anthony, T. Goldstein, K. J. Olival, M. M. Doyle, N. R. Gardner, B. Bird, W. A. Smith, D. Wolking, K. Gilardi, C. Monagin, T. Kelly, M. Uhart, J. H. Epstein, C. Machalaba, M. K. Rostal, P. Dawson, E. Hagan, A. Sullivan, H. Li, A. A. Chmura, A. Latinne, C. Lange, T. O'Rourke, S. H. Olson, L. Keatts, A. P. Mendoza, A. Perez, C. Dejuste de Paula, D. Zimmerman, M. Valitutto, M. LeBreton, D. McIver, A. Islam, V. Duong, M. Mouiche, Z. Shi, P. Mulembakani, C. Kumakamba, M. Ali, N. Kebede, U. Tamoufe, S. Bel-Nono, A. Camara, J. Pamungkas, K. Coulibaly, E. Abu-Basha, J. Kamau, S. Silithammavong, J. Desmond, T. Hughes, E. Shiilegdamba, O. Aung, D. Karmacharya, J. Nziza, D. Ndiaye, A. Gbakima, Z. Sijali, S. Wacharapluesadee, E. Alandia Robles, B. Ssebide, G. Suzán, L. F. Aguirre, M. R. Solorio, T. N. Dhole, N. T. T. Nga, P. L. Hitchens, D. O. Joly, K. Saylors, A. Fine, S. Murray, W. Karesh, P. Daszak, J. A. K. Mazet, PREDICT Consortium, C. Dejuste de Paula, C. K. Johnson.
Commun Biol. 2022;5(1):844.

11. Cambodia achieved a high vaccination coverage for its population: A good example of a lower middle-income country

Ngoc Phuong Hong Tao, Dang Nguyen, Le Huu Nhat Minh, Veasna Duong, Chey Beaupha, Tareq Al Ahdal, Nguyen Tien Huy.
J Glob Health. 2022;12:03088.

12. Robust and Functional Immune Memory Up to 9 Months After SARS-CoV-2 Infection: A Southeast Asian Longitudinal Cohort

Hoa Thi My Vo*, Alvino Maestri*, Heidi Auerswald, Sopheak Sorn, Sokchea Lay, Heng Seng, Sotheary Sann, Nisa Ya, Polidy Pean, Philippe Dussart, Olivier Schwartz, Sovann Ly, Timothée Bruel, Sowath Ly, Veasna Duong, Erik A. Karlsson**, Tineke Cantaert**.
Frontiers in Immunology [En ligne]. 2022 [cité le 1 mars 2022];13.

13. **Temporal patterns of functional anti-dengue antibodies in dengue infected individuals with different disease outcome or infection history.**
    Hoa Thi My Vo, Vinit Upasani, Heidi Auerswald, Sokchea Lay, Sotheary Sann, Axelle Vanderlinden, Sreymom Ken, Sopheak Sorn, Sowath Ly, Veasna Duong, Philippe Dussart, Tineke Cantaert.
    Sci Rep. Nature Publishing Group; 2022;12(1):17863.

14. **Household clustering supports a novel chemoprophylaxis trial design for a mosquito-borne viral disease**
    Hugh R. Watson, Veasna Duong, Sowath Ly, Marie Mandron, André M. Siqueira, Guilherme S. Ribeiro.
    Int J Infect Dis. 2022;122:169-73.
15. **Presence of Recombinant Bat Coronavirus GCCDC1 in Cambodian Bats**
    Feng Zhu*, Veasna Duong*, Xiao Fang Lim, Vibol Hul, Tanu Chawla, Lucy Keatts, Tracey Goldstein, Alexandre Hassanin, Vuong Tan Tu, Philippe Buchy, October M. Sessions, Lin-Fa Wang, Philippe Dussart, Danielle E. Anderson.
    Viruses. 2022;14(2):176.

16. **Childhood encephalitis in the Greater Mekong region (the SouthEast Asia Encephalitis Project): a multicentre prospective study**
    Jean David Pommier, Chris Gorman, Yoann Crabol, Kevin Bleakley, Heng Sothy, Ky Santy, Huong Thi Thu Tran, Lam Van Nguyen, Em Bunnakea, Chaw Su Hlaing, Aye Mya Min Aye, Julien Cappelle, Magali Herrant, Patrice Piola, Bruno Rosset, Veronique Chevalier, Arnaud Tarantola, Mey Channa, Jerome Honnorat, Anne Laure Pinto, Sayaphet Rattanavong, Manivanh Vongsouvath, Mayfong Mayxay, Sommanikhone Phangmanixay, Khounthavy Phongsavath, Ommar Swe Tin, Latt Latt Kyaw, Htay Htay Tin, Kyaw Linn, Thi Mai Hung Tran, Philippe Pérot, Nguyen Thi Thu Thuy, Nguyen Hien, Phuc Huu Phan, Philippe Buchy, Philippe Dussart, Denis Laurent, Marc Eloit, Audrey Dubot-Pérès, Olivier Lortholary, Xavier de Lamballerie, Paul N Newton, Marc Lecuit; SEAe Consortium
    Lancet Glob Health. 2022 Jul;10(7):e989-e1002.

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. Sébastien Boyer by Institut Pasteur in Paris for a permanent position.

Compared to the last two years, the Unit's staff has increased to a peak of 18 people for a period of six months. As of 1 January 2023, the Medical and Veterinary Entomology Unit has 17 members.

Three foreign scientists (Antsa Rakotonirina, Pierre-Olivier Maquart 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. A second PhD student, Sim Mala, had begun in October 2022 but resigned in December 2022 for personal reasons.

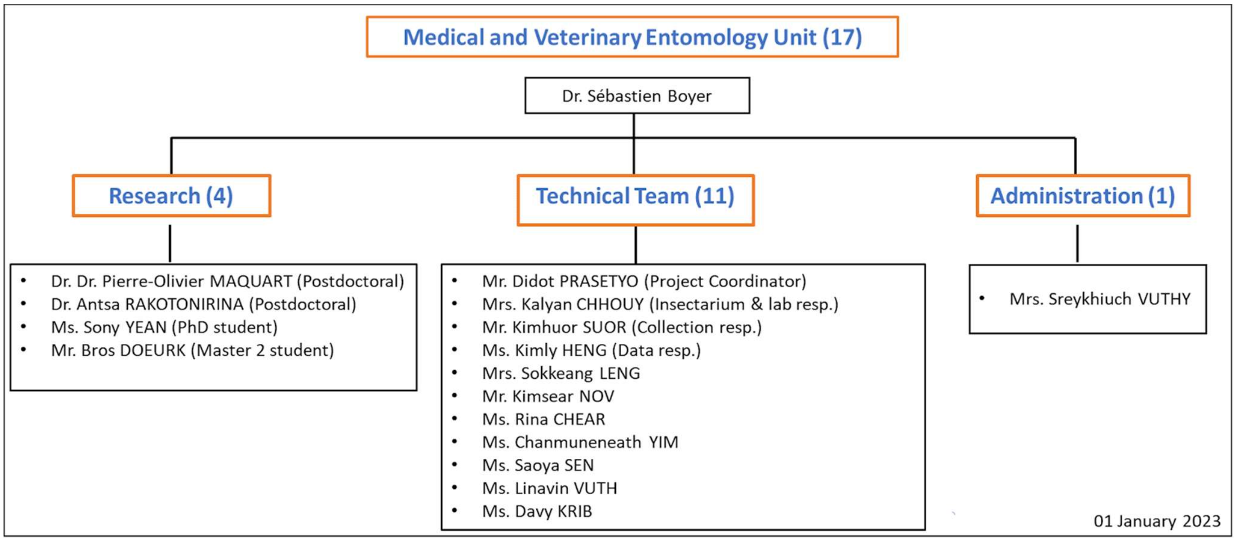


Figure 24. Medical & Veterinary Entomology Unit Organigram

The arrival of four new technical staff in 2022 aimed to have a better distribution of our personnel across our various projects. This year, as detailed below, we have managed to balance our number of field missions, and to develop more laboratory activities. We have also begun the development of Veterinary Entomology capabilities, while continuing to expand Medical Entomology.

4.5.2 Major Achievements in 2022

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

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

The Ecomore 2 Project is funded by the AFD (*Agence Française de Développement*). The project's objective is to determine whether a successful integrated vector management system in localized areas could decrease the incidence of dengue virus in rural and peri-urban communities. The project will officially conclude in June 2023. A technical audit and a steering committee review were conducted during the year 2022.

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

DARPA-PREEMPT Project: Preventing Emerging Pathogenic Threats.

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

In 2022, there were no field sampling missions and no laboratory experiments. During the year, we published one article on a DARPA project (Maquart et al. 2022, on mangrove areas), and submitted another in December 2022 (Rakotonirina et al., on forested areas).

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

The Medical and Veterinary Entomology Unit's role in this project has now concluded.

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

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

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

The field missions and main laboratory experiments have now ended. The final technical and financial reports were sent to the French Foreign Ministry for Europe (MEAE) in March 2021.
The four items below are in progress, and more may follow:

- factors influencing the distribution of mosquito species along an ecological gradient from forest to city;
- description of new viruses and their evolutionary relationship with mosquito species;
- distribution of viruses within mosquito species within an ecological gradient from forest to city;
- potential arboviruses cultivated on both mosquitos and human cells.

Some of these analyses are in progress, and a few PCR remain to be done. Some of them are being done by our collaborators at *Institut Pasteur* in Paris, and some others by our Unit and also by the Virology Unit.

Start–End Years	*2019-2021*
Collaborations	Institut Pasteur Paris Ministry of Health Wildlife Conservation Society IRD (GeoHealth)
Funding	FSPI 2019-17

Mosquito Species and Dynamics in Phnom Penh.

The diversity, distribution and seasonality of mosquito species in Phnom Penh is not yet fully known. A study on the dynamics of dengue vectors in Phnom Penh was undertaken in 2019 and 2020. The relative abundance of the different species is being analyzed against different meteorological parameters, and the different types of urban environments surrounding pagodas. The sampling was done around pagodas since in the rapidly urbanizing Cambodian capital, pagodas will be left undeveloped and unchanged in the near future, allowing replication of this study over several decades and providing important time-series data. The objective will be to evaluate the dynamics, and the risk associated with the potential presence of mosquito vector species.
The field missions began in 2019. The sampling was done in forty different sampling points in Phnom Penh Pagodas between March 2019 and March 2020. During this period, 9,054 adult mosquitoes were collected, including 5,080 *Ae. aegypti* and 2,771 *Ae. albopictus*. These two dengue vector mosquito species represent 87% of all the mosquitoes sampled during the year. Their distribution in Phnom Penh shows that *Ae. aegypti* is more common in high human density areas, whereas *Ae. albopictus* is more likely to be the species found in more wooded areas.

Based on this internal IPC project, a collaborative project based on our design and sampling was designed with Michael C. Fontaine from MiVEGEC (IRD). This project aimed to explain, and date, the arrival of *Ae. albopictus*. This project (MUSE INVALBO) was funded in 2021.The characterization of *Ae. albopictus* invasivity in Phnom Penh will be completed in 2023. We will characterize the genomic diversity, the genetic structuring of populations, the dispersal and flow of genes along the anthropization gradients and the genetic inference from recent demographic history and its evolution to determine the factors of invasion in urban environments in Phnom Penh and Europe. Using samples obtained via FSPI projects (in forests) and in Phnom Penh, *Ae. albopictus* will be analyzed by Dr. Michael Fontaine, IRD, Montpellier.

Several articles were written with this data. One article was published in 2021 on the presence of *Ae. albopictus* - that had not previously been detected - in Phnom Penh. Two other articles were published in 2022 on the predicted distribution of these arbovirus vector species in Southeast Asia, based in part on this data (Bonnin et al. 2022), and on a statistical analysis conducted to study invasive mosquitoes (Da Re et al., 2022).

Two articles are planned for 2023: one article on the factors explaining the distribution of dengue vector species in Phnom Penh by Pierre-Olivier Maquart, and another on the history of *Aedes albopictus* invasions, by Michael Fontaine (IRD).

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

Collaborations	Ministry of Cults and Religion, Cambodia Ministry of Tourism, Cambodia Mivegec, IRD, France Entropie, IRD, Univ Réunion, CNRS, Ifremer, New Caledonia UCLouvain, Belgium
Funding	IPC: 2019-2023 KIMRIVE – MUSE 2021

NIH PICREID Project: Pasteur Institute – 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 2022, 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.

In 2021 and 2022, we have collected 52,106 mosquitoes. Among the collected mosquitoes, 17,587 mosquitoes from 1,862 pools were sent to *IP* Paris for virus discovery.

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

Wat-Health Project (FSPI project).

This project studies the effects of anthropological factors on complex changes in mosquitoes and viruses. The Wat-Health project will help to determine the connection between flooding and mosquito populations, pollutants, and sanitary risks in Cambodia. Our unit's participation in the project is focused on defining the relationship between flooding events and mosquito populations, and evaluating the indirect sanitary risks in the studied area. We will also evaluate resistance to insecticides.

This project was approved in 2021 and started in April 2021. The field missions stopped in September 2022, after the completion of 15 field missions. Overall, 8,595 mosquitoes were collected, using 15 light-traps for three consecutive days every month. Larvae were also collected for the identification of breeding sites for the most representative species, and to initiate bioassays to determine the level of insecticide resistance at this specific location. The data analysis is to be conducted by the project leader and the other relevant partners.

Collaborations	Ambassade de France au Cambodge, Cambodia IRD (Ecoland, GeoHealth) Ministry of Agriculture, Cambodia Institut Technologique du Cambodge (ITC), Cambodia Royal University of Agriculture (RUA), Cambodia Institut Pasteur de Nouvelle Calédonie (IPNC), New Caledonia, France
Funding	Fonds de solidarité pour les projets innovants, Paris, France

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

The project will analyze the mechanisms underlying the impact of biodiversity on the risk of infectious disease emergence and aims to define tools of context-adapted biodiversity conservation and restoration strategies to reduce zoonotic risk. 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 via this project 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 will begin in 2022, starting with the recruitment of dedicated technicians.

Collaborations	CIRAD (Julien CAPPELLE) Virology Unit Ministry of Environment, Cambodia Institut Technologique du Cambodge (ITC), Cambodia Royal University of Phnom Penh, Cambodia
Funding	French National Research Agency (ANR- Agence nationale de la recherche)

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

The preliminary data 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. Moreover, it provided interesting perspectives on the use of this technique as an inexpensive tool for exploring the immune response of arbovirus-infected mosquitoes.

Collaborations	Virology Unit Institut Pasteur of Korea
Funding	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, a second-year master's degree student 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 densities 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.

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

NOSI-TICK.

The Principal Investigator is Dr. NOUHIN Janin (Virology Unit), with Dr. Pierre-Olivier Maquart as co-PI. This project is nested within an NIH-funded project, the *Inter-Regional Study of Transmission, Adaptation and Pathogenesis of Viruses with Pandemic Potential in Southeast Asia and West/Central Africa*, also known as the PICREID project (U01 AI51758-01). The findings will provide relevant data which will be useful for the diagnosis and appropriate treatment of tick-borne pathogens, and for the assessment of host–vector interaction by enabling the documentation of previous exposures to ticks and tick-borne pathogens.

The present study focuses on tick diversity and on their endofauna, to bring to light tick-borne diseases and their transmission vectors in rural Cambodia. We aim to identify tick species and associated pathogens, including viruses and bacteria circulating in Cambodia. Additionally, we aim to investigate the transmission risks of identified tick-borne pathogens in humans, by assessing seroprevalence (IgG) and tick bite biomarkers in people living in the communities where ticks are captured.

Tick collections will be planned according to the parent study. The collected tick specimens will be first identified morphologically and confirmed using MALDI-TOF MS. Once identified, the specimens will be kept at -80°C. Ticks samples will be tested for known viruses and bacteria using molecular assays, and for novel pathogens using metagenomics sequencing. Human samples will be used for seroprevalence study of commonly known tick-borne viruses and bacteria circulating in Asia, and for

tick bite biomarkers. Institut Pasteur du Cambodge will perform all of the tasks stated above in collaboration with Institut Pasteur in Paris.
In the context of our tick-bite biomarker assessment, we will rely on our database of previously identified molecules present in the saliva of Ixodes ricinus. First, we will evaluate the protein candidates with animal serum and ticks collected in Cambodia. The experiments will be conducted at Institut Pasteur du Cambodge and Institut Pasteur in Paris. For this purpose, parts of animal serum and tick samples will be shipped to Dr. Sarah BONNET, Institut Pasteur in Paris.

Our study will provide knowledge on the current state of tick populations and on the potential risks of tick-associated pathogens circulating in Cambodia.

Collaborations	Virology Unit, Instut Pasteur du Cambodge Institut Pasteur, Paris
Funding	Medical and Veterinary Entomology Unit

Southeast Asia Tick Identification Key (SEA TICKEY).
This project began in April 2022 and will 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, and the Ministry of Agriculture. This project was funded by a FSPI dedicated to regional One Health projects in Southeast Asia.

We propose to create and develop 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 TBDs in humans and livestock at the national level. It will also allow the development of several planned research projects on TBDs 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, Cambodia IRD (Ecoland, GeoHealth) University of Hokkaido, Japan Institut Pasteur, Paris
Funding	Regional FSPI - Fonds de solidarité pour les projets innovants, Paris, France

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 (URA) 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, Cambodia IRD (Ecoland, GeoHealth) University of Hokkaido, Japan Institut Pasteur, Paris
Funding	Fonds de solidarité pour les projets innovants, Paris, France

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:

- 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

The barcoding of COI genes is in progress. Up until now, 111 mosquitoes belonging to 24 species have been barcoded, or sequenced.

- 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 60 spectra of one species (*Rhipicephalus sanguineus*). More species will be added in the future.

- 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 2023.

- 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.

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 ZooCoV and BCOMING for ectoparasites samples Medical and Veterinary Entomology Unit for MALDI-TOF MS and Barcoding

4.5.3 Research Programs- Outlook for 2023

In 2021, the following written and proposed scientific projects were not funded: IKI-KARST (GIZ call), MALDI-RDT (post-doctoral PICREID call) and MODEM (ACIP call).

Preventing Zoonotic Diseases Emergence (PREZODE)

Througn 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 and reinforce surveillance to work towards an integrated One Health system.

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 above) has for objective to develop an identification key for tick species.

4.5.5 Teaching and Training

Mentorship

Master's Students

Mr. Doeurk Bros (Master's 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"

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"

Teaching

Since 2020, Sébastien 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".

06-10 June 2022	**Taxonomy and Biology of Ticks: Tick Collection in the Field** Trainer: Dr. Sarah BONNET, *Institut Pasteur*, France Trainees: 4 staff from IPC, 4 from the Ministry of Agriculture, and from RUA
06-10 June 2022	**Molecular Identification of Mosquitoes** Trainer: Dr. Mallorie HIDE, IRD Trainees: 2 postdoctoral students, 3 technicians
20-22 July 2022	**First Aid and Immobilization** Trainer: MC & Co Prevention Trainees: IPC staff, including 4 from Medical and Veterinary Entomology
27-30 September 2022	**Mapping the Risk: qGIS Training** Trainer: GeoHealth, IRD Trainees: 4 staff from IPC, 4 from the Ministry of Agriculture, and 4 from RUA
13 October 2022	**Taxonomy of the Main Ectoparasites Genera** Trainer: Didot PRASETYO Trainees: all technical staff from the Medical and Veterinary Entomology Unit
October 2022	**Mosquito Biology, Taxonomy, Mounting of Larvae and Dissection of Genitalia** Trainer: Gilbert LE GOFF, IRD, France Trainees: all technical staff from the Medical and Veterinary Entomology Unit
29 November 2022	**Taxonomy Training for Tick Genus Determination** Trainer: Didot PRASETYO Trainees: all technical staff from the Medical and Veterinary Entomology Unit
28 November – 02 December 2022	**Mapping and Spatial Analysis in R for One Health Studies** Trainer: GeoHealth, IRD Trainees: 1 postdoctoral student
12-16 December 2022	**Taxonomy and Biology of Ticks: Tick Collection in the Field** Trainer: Dr. Sarah BONNET, *Institut Pasteur*, France Trainees: 4 staff from IPC, 4 from the Ministry of Agriculture, and 4 from RUA

Calmet & Yersin Grant

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

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

4.5.6 Outlook

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 Publications List in 2022

NOTE

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

1. **Culex vishnui Theobald, 1901 (Diptera: Culicidae): an Overlooked Vector in South-East Asia**
Pierre-Olivier Maquart, Leakena Chann, Sébastien Boyer
J Med Entomol. 2022 Jul;59(4):1144-1153. DOI: 10.1093/jme/tjac044. PMID: 35522221

2. **Culex vishnui**
Pierre-Olivier Maquart, Sébastien Boyer
Trends Parasit. 2022 Jun;38(6):491-492. DOI: 10.1016/j.pt.2022.01.003. PMID: 35153157

3. **Xenopsylla cheopis (Rat Flea)**
Sébastien Boyer, Thomas R. Gillespie, Adélaïde Miarinjara
Trends Parasitol. 2022 Jul;38(7):607-608. DOI: 10.1016/j.pt.2022.03.006. PMID: 35527197

4. **Plastic pollution and infectious diseases.**
Pierre-Olivier Maquart, Yves Froehlich, Sébastien Boyer
The Lancet Planetary Health, 2022;6(10):e842-e845. DOI: 10.1016/S2542-5196(22)00198-X

5. **DynamAedes: a unified modelling framework for invasive Aedes mosquitoes.**
Daniele Da Re, Wim Van Bortel, Friederike Reuss, Ruth Müller, Sébastien Boyer, Fabrizio Montarsi, Silvia Ciocchetta, Daniele Arnoldi, Giovanni Marini, Annapaola Rizzoli, Gregory

L'Ambert, Guillaume Lacour, Constantianus J. M. Koenraadt, Sophie O. Vanwambeke & Matteo Marcantonio
Parasit. Vectors; 2022 Nov;15(1):414. doi: 10.1186/s13071-022-05414-4. PMID: 36348368

6. **Predicting the continental-scale effects of climate change on arbovirus vectors density in Southeast Asia through process-based modelling.**
   Lucas Bonnin, Annelise Tran, Vincent Herbreteau, Sébastien Marcombe, Sébastien Boyer, Morgan Mangeas, Christophe Menkes
   Environ. Health Perspect. 2022 Dec; 130(12):127002. DOI: 10.1289/EHP11068. PMID: 36473499

7. **MALDI-TOF MS: an effective tool for a global surveillance of dengue vector species.**
   Antsa Rakotonirina, Morgane Pol, Fara Nantenaina Raharimalala, Valentine Ballan, Malia Kainiu, Sébastien Boyer, Sosiasi Kilama, Sébastien Marcombe, Sylvie Russet, Emilie Barsac, Rama Vineshwaran, Malia Kaleméli Selemago, Vincent Jessop, Geneviève Robic, Romain Girod, Paul T. Brey, Julien Colot, Myrielle Dupont-Rouzeyrol, Vincent Richard, Nicolas Pocquet
   PLOS One, 2022 Oct;17(10):e0276488. DOI: 10.1371/journal.pone.0276488 PMID: 36264911

8. **Mosquito diversity (Diptera: Culicidae) and medical importance in Koh Kong mangrove forests (Cambodia).**
   Pierre-Olivier Maquart, Chea Sokha, Sébastien Boyer
   Asian Biomed (Res Rev News) 2022;16(3):121–129. DOI: 10.2478/abm-2022-0015

9. **Zika vector competence data reveals risks of outbreaks: the contribution of the European ZIKAlliance project.**
   Thomas Obadia, Gladys Gutierrez-Bugallo, Veasna Duong, Ana I. Nuñez, Rosilainy S. Fernandes, Basile Kamgang, Liza Hery, Yann Gomard, Sandra R. Abbo, Davy Jiolle, Uros Glavinic, Myrielle Dupont-Rouzeyrol, Célestine M. Atyame, Nicolas Pocquet, Sébastien Boyer, Catherine Dauga, Marie Vazeille, André Yébakima, Michael T. White, Constantianus J. M. Koenraadt, Patrick Mavingui, Anubis Vega-Rua, Eva Veronesi, Gorben P. Pijlman, Christophe Paupy, Núria Busquets, Ricardo Lourenço-de-Oliveira, Xavier De Lamballerie, Anna-Bella Failloux.
   Nat Commun. 2022 Aug 2;13(1):4490. doi: 10.1038/s41467-022-32234-y. PMID: 35918360

10. **Small scale field assessment against the dengue vector Aedes aegypti using the auto-dissemination approach in an urban area of Vientiane, Lao PDR.**
    Phoutmany Thammavong, Sebastien Boyer, Phonesavanh Luangamath, Nothasine Phommavanh, Vaekey Vungkyly, Somphat Nilaxay, Khaithong Lakeomany, Paul Brey, Marc Grandadam, Sebastien Marcombe
    PLOS One. 2022 Jul 1;17(7):e0270987. DOI: 10.1371/journal.pone.0270987. PMID: 35776762

11. **Genomic shifts, phenotypic clines and fitness costs associated with cold tolerance in the Asian tiger mosquito.**
    Stéphanie Sherpa, Jordan Tutagata, Thierry Gaude, Frédéric Laporte, Shinji Kasai, Intan H. Ishak, Xiang Guo, Jiyeong Shin, Sébastien Boyer, Sébastien Marcombe, Theeraphap Chareonviriyaphap, Jean-Philippe David, Xiao-Guang Chen, Xiaohong Zhou, Laurence Després.
    Mol Biol Evol. 2022 May 3;39(5):msac104. DOI: 10.1093/molbev/msac104. PMID: 35574643

12. **Monitoring insecticide resistance of adults and larvae Aedes aegypti (Diptera: Culicidae) in Phnom Penh, Cambodia.**
    Sébastien Boyer, Pierre-Olivier Maquart, Kalyan Chhuoy, Kimhuor Suor, Moeun Chhum, Kimly Heng, Sokkeang Leng, Didier Fontenille, Sébastien Marcombe
    Parasit Vectors. 2022 Jan 31;15(1):44. DOI: 10.1186/s13071-022-05156-3. PMID: 35101104

13. **High Rickettsial diversity in rodents and their ectoparasites from the central highlands of Madagascar**
    Fanohinjanaharinirina Rasoamalala, Mamionah N J Parany, Soloandry Rahajandraibe, Malala N Rakotomanga, Tojo Ramihangihajason, Voahangy Soarimalala, Sébastien Boyer, Minoarisoa Rajerison, Beza Ramasindrazana
    J Med Entomol. 2022 Mar 16;59(2):667-674. DOI: 10.1093/jme/tjab207. PMID: 34958102

14. **Variations in carapace shape of Whip Spiders (Arachnida: Amblypygi)**
    Florian Réveillion, Sophie Montuire, Pierre-Olivier Maquart, Charlotte Fétiveau, Loïc Bollache.
    Journal of Morphology, 2022 283(8):1-12. DOI: 10.1002/jmor.21485.

4.6 Medical Biology Laboratory

4.6.1 Functional Structure

The Medical Biology Laboratory (MBL) provides a platform for comprehensive biological analyses. A range of approximately 150 tests is offered to public/private hospitals and clinics, non-governmental organizations (NGOs), and walk-in patients.

The MBL's staff includes 34 people belonging to seven divisions: (1) the reception area, (2) the sampling area, (3) the microbiology laboratory, (4) the mycobacteriology laboratory, (5) the blood biology laboratory, (6) the molecular biology platform and (7) the Bacteriology Research Laboratory (LMI DRISA / International Joint Laboratory – Drug Resistance in Southeast Asia).

A 4-year ISO 15189 accreditation had been granted in 2018 for biochemistry, hematology and microbiology. In 2022, the renewal of this accreditation by the Comité Français d'Accréditation (COFRAC) has been announced for a period of 5 years (01/12/2022 - 31/11/2027).

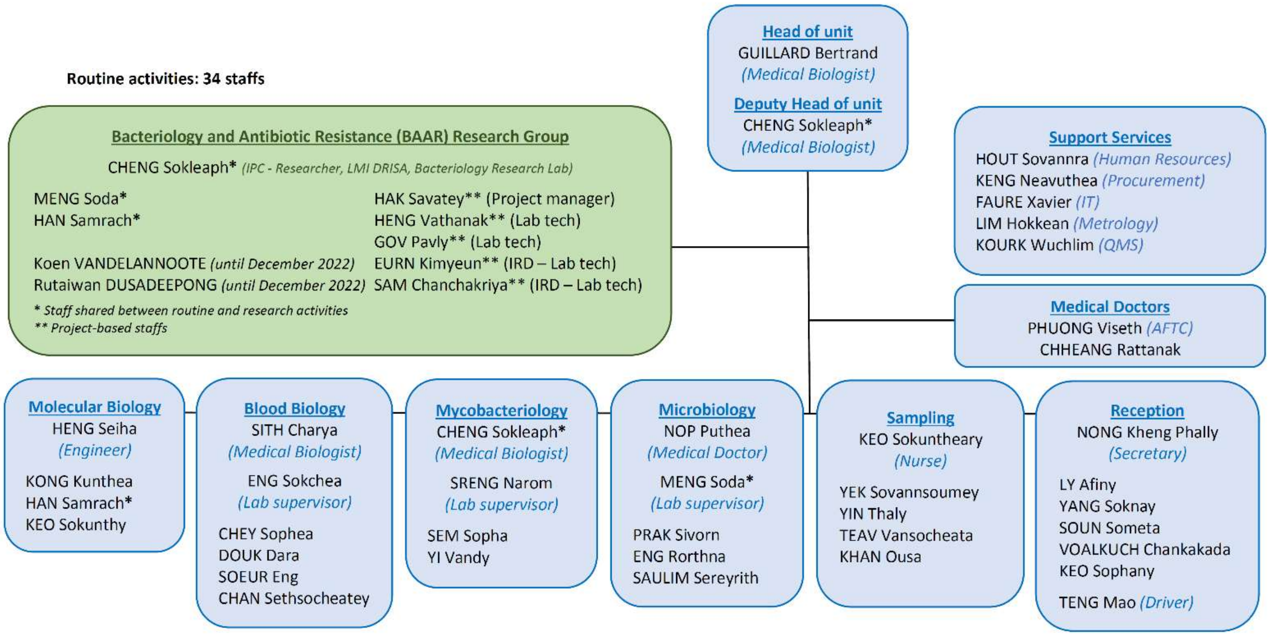


Figure 25. Organogram - Medical Biology Laboratory (January 2023)

4.6.2 Service Activities in 2022

The Medical Biology Laboratory performed 161 500 analyses in 2022, which represents 8.3 million B, an increase of 38.6 % compared to 2021. (To evaluate the Laboratory's activity levels, analyses and tests are expressed in "key-letter B" units, according to the French Nomenclature for Medical Biology Analyses: NGAP) The year 2022 has seen a rapid decline in COVID-19 testing activity and a resumption of routine activities. However, COVID-19-related activities still represented 53.6% of the MBL's total income in 2022 (56.5% in 2021). Figure 26 illustrates the trend of our activities over the last 15 years. The IPC's MBL remains a reference in Cambodia, despite an evolving medical biology sector that is seeing the establishment of new private laboratories in Phnom Penh, and with health service providers who were previously sending their samples to MBL now setting up internal laboratories.

Nevertheless, the MBL is regularly solicited for its microbiology and molecular biology services, and to verify some pathological blood results obtained in other laboratories.

In 2022, the total activity of the molecular biology platform has continued to increase (+28.5 % compared to 2021), particularly for activities not linked to COVID-19, which have increased by 52.7 % compared to 2021.

Evolution of LBM Activity (Number of 8)

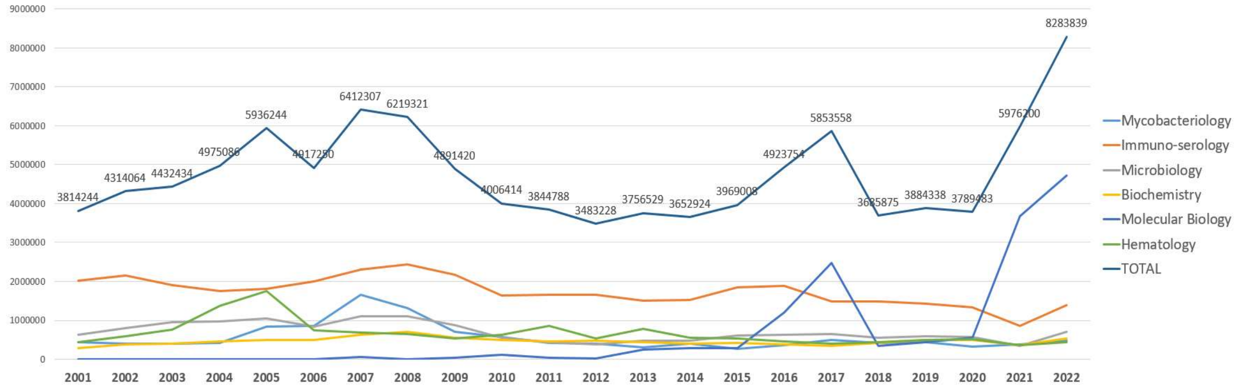


Figure 26. Trends in MBL activities expressed in B (list of reimbursed biological procedures, format adapted for the IPC's MBL using the French Nomenclature for Medical Biology Acts, or NABM, values expressed in key-letter B).

4.6.3 HIV

Voluntary Confidential Counselling and Testing for HIV (VCCT)

In 2022, 454 patients consulted the VCCT and benefited from a free HIV consultation and screening (Figure XX). HIV-positive patients represented 47.8 % of the consultations. This high rate of positivity is due to the fact that most patients were referred by other health centers for a free confirmation of a positive HIV antibody rapid test administered elsewhere. Of the 454 patients, 191 were referred by NGOs (Men's Health Cambodia, Reproductive Health Association of Cambodia, Chouk Sor Clinic) working with the most at-risk populations, such as entertainment workers, men who have sex with men and transgender people. In addition, 64 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 centers focused on the most at-risk populations. On the other hand, the MBL's VCCT activities remain the reference for these centers to confirm their results.

HIV Nominative Serology (MBL patients)

We observed a 31.7 % increase in the number of HIV serology screenings compared to 2021 (after a significant decrease during the COVID-19 pandemic).

In 2022, the seropositivity rate of MBL patients remained stable at 4.1 % (Figure 27). The prevalence is higher than the general population because a part of the samples were sent by other laboratories to confirm their positive results.

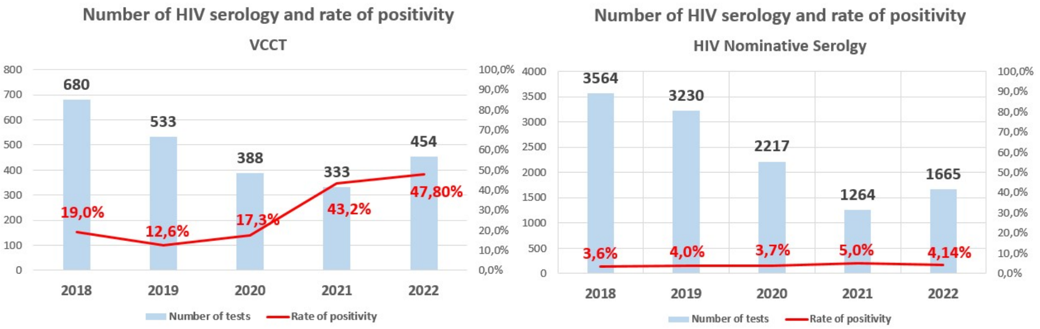


Figure 27. HIV serology and rate of positivity at VCCT and MBL

4.6.4 Bacteriology

Following a major drop linked to the COVID-19 crisis, the number of bacterial cultures doubled in 2022. We observed a 6 % increase in the rate of extended-spectrum-beta-lactamase (ESBL) Enterobacteriaceae (34 %) in comparison with 2021, while Carbapenemase-producing Enterobacteriaceae (CPE) decreased (-1.3 %), mainly within Escherichia coli species. Methicillin-resistant Staphylococcus aureus (MRSA) increased by 4 %, and Carbapenem-resistant Acinetobacter baumannii (CRAB) decreased significantly by 14 %. To date, no resistance to vancomycin has been detected in Enterococcus faecium. Finally, 15 strains of Burkholderia pseudomallei were isolated (Table 4).

Table 4. Bacteriological data in 2022

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

In 2022, as an alternative to the classic bacterial culture for the diagnosis of sexually transmitted infections (STIs), the MBL performed 273 Chlamydia trachomatis/Neisseria gonorrhoeae PCRs on vaginal, urine, urethral, throat, and anal samples. Of these, 9.2 % (n = 25) were positive for Chlamydia trachomatis, and 5.5 % (n = 15) for Neisseria gonorrhoeae, while we detected 6 co-infections (2.2 %).

4.6.5 COVID-19

In 2022, the MBL carried out 21,311 COVID-19 RT-PCR tests (16,873 in 2021, +26%) (Figure 28). COVID-19 tests for travellers represented 84.0 % of COVID-19-related activities. However, there was a rapid decline in COVID-19 testing activities during the second half of the year, mainly due to the COVID-19-negative test requirement being waived for international travel by many countries.

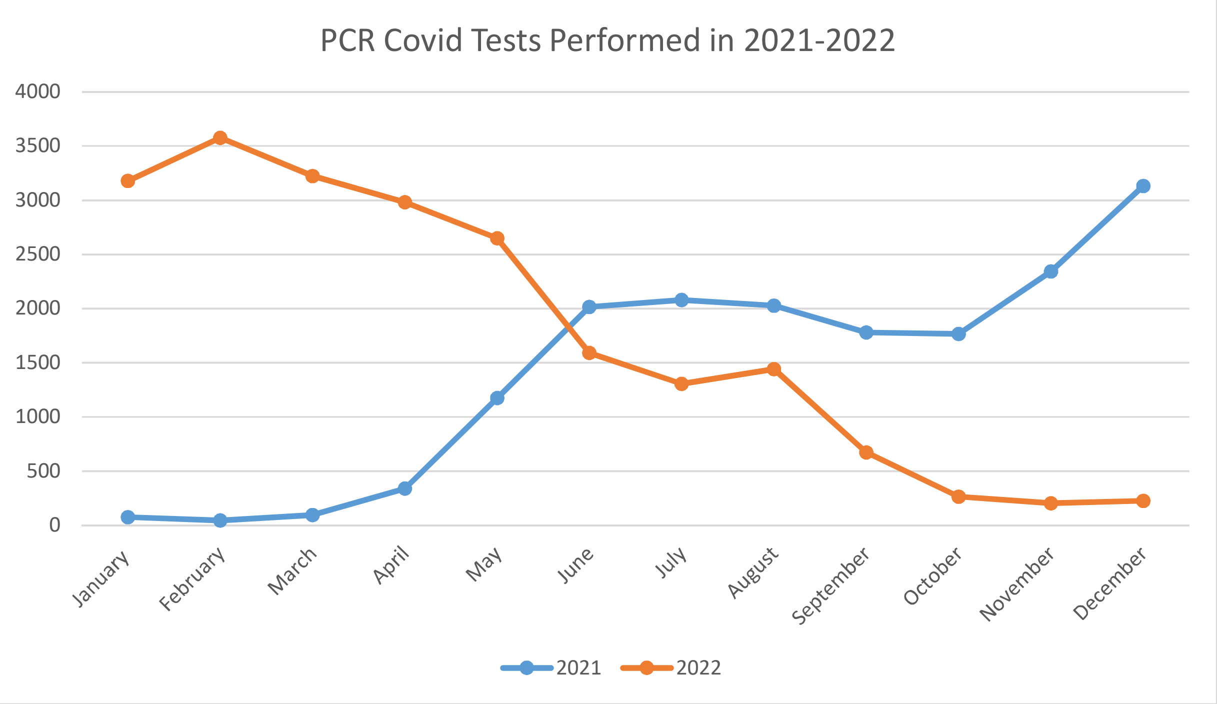


Figure 28. COVID PCR Activities, 2021-2022

4.6.6 Tuberculosis

Mycobacteriology activities have significantly increased after the COVID-19 pandemic. The total number of analyses involving mycobacteriology was 7082 in 2022, representing an increase of 23.8 % compared to the number of analyses in 2021 (5719). This increase is due to the uptake of TB screening activities using the TB rapid diagnosis with the GeneXpert system. In 2022, the number of Xpert assays performed doubled compared to the previous year. 1707 Xpert MTB/RIF Ultra tests were performed for rapid TB diagnosis, of which 23.6 % were MTBC positive, and 3.1 % of positive samples were resistant to Rifampicin. Figure 29 shows the detection rates for M.tb and RIF resistance by Xpert MTB/RIF Ultra assay over the last five years.

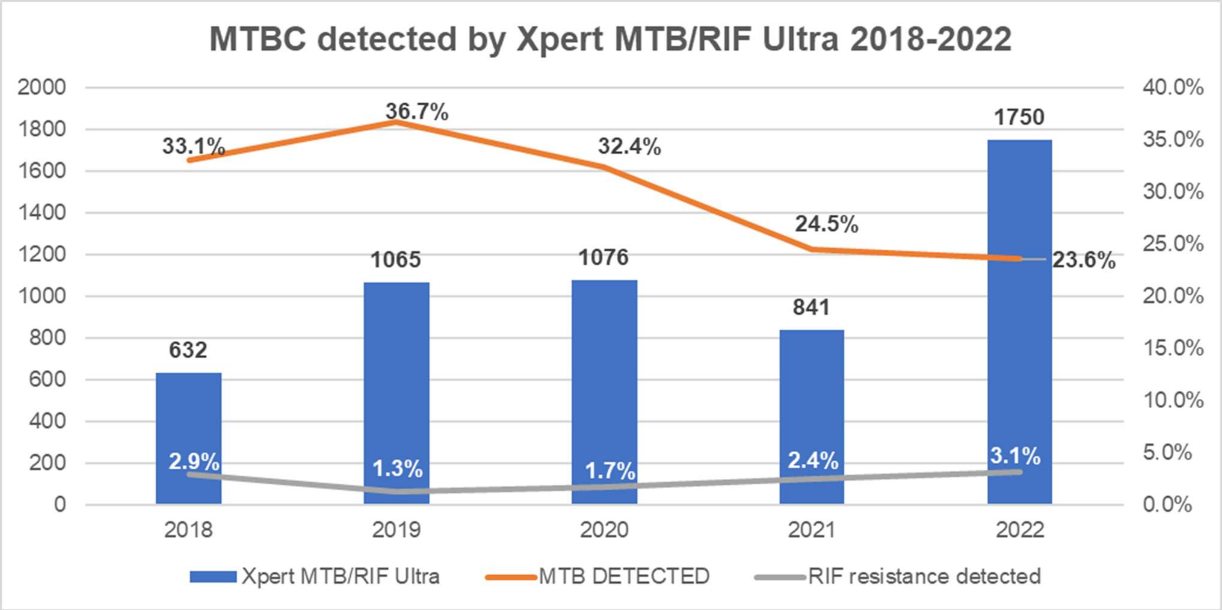


Figure 29. GeneXpert Xpert MTB/RIF Activity 2018-2022

4.6.7 Research Programs – Major Achievements in 2022

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

This is a continuing project from LMI-DRISA that was set up in 2016 as part of a regional effort with Vietnam, Cambodia and Lao PDR. The main objective of the LMI is to share competencies and best practices between different academic and non-academic institutions in these three countries and in France, in order to study the mechanisms and factors that influence 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 (2020-2025), named 'LMI-DRISAOH' aims to integrate a 'One Health' approach to unravel what is truly happening for antimicrobial resistance emergence and spread at the human/animal/environment interface.

Collaborations	Team Leader: S. CHENG (MBL), T.K.O. NGUYEN (USTH), Q.H. NGUYEN (USTH) and A.L. BAÑULS (IRD) MIVEGEC Unit, IRD (M. HIDE) Medical Biology Laboratory, IPC (G. DELVALLEZ) Centre d'Infectiologie Lao Christophe Mérieux (CILM) Fondation Mérieux, National Institute of Hygiene and Epidemiology (NIHE) University of Science and Technology of Hanoi (USTH) Oxford University Clinical research (OUCRU)
Funding	IRD (2020-2025)

Antibiotic Resistance at the Human/Animal/Environment Interface in a "ONE-HEALTH" Approach in Cambodia: ARCAHE

The emergence and spread of antibioresistance (ABR) are mostly attributed to the overuse of antibiotics, whether in humans, in animals or in the environment. The main objective of the project is to explore the circulation of antibiotic-resistant bacteria at the interface between humans, animals and the environment in Cambodia. To achieve the objectives, a total of 355 patients were recruited from two hospitals in Cambodia (129 in the Battambang Provincial Referral Hospital and 226 in Calmette Hospital), and 1869 samples were collected from the patients' close environments (including 980 animal samples, 601 environmental samples, and 288 food and water samples). Among the studied patients, the three main bacterial species identified were Escherichia coli (EC; 48.5 %), Staphylococcus aureus (SA; 23%) and Klebsiella pneumoniae (KP; 12%) and 58.7 % of them were multidrug-resistant bacteria. Of the samples collected from the patients' close environments, 939 samples underwent bacteriological analysis for the detection of multidrug-resistant bacteria, and such multidrug-resistant bacteria were isolated in 18.1 % of the animal samples, 54.5 % of the environmental samples, and 68.1 % of the drinking water and food samples, for a total of 495 cases. In terms of bacterial species, the 3 main bacterial species detected were EC (n=265; 53.5 %), KP (n=163; 32.9 %) and Enterobacter cloacae complex (n=43; 8.7 %). The data collection and bacteriology analysis were finished in 2021. Our activities in 2022 were mainly focused on NGS and the analysis of 539 selected bacteria isolates and 751 samples from patients' environments. Overall, 788 sequencings were performed in 2022. A further 394 sequencings and the following bioinformatic analyses are expected to be finished in 2023. A two-week training on bioinformatics was conducted for one local researcher who will participate in the bioinformatic analyses.

Collaborations	Team Leaders: S. CHENG (MBL) and AL. BAÑULS (IRD) MIVEGEC Unit, IRD (M. HIDE, J. HAYER) ESPACE-DEV Unit, IRD (V. HERBRETEAU) Medical Biology Laboratory (G. DELVALLEZ), Epidemiology and Public Health Unit (P. PIOLA and C. FLAMANT), Calmette Hospital (S. BORY), Battambang provincial Referral Hospital (S. CHIEK), CIRAD (V. CHEVALIER), Laboratory of Environment and Food Safety, IPC (N. SRENG), General Directorate of Animal Health and Production (GDAPH), MAFF (S. SAN)
Funding	Fonds de solidarité pour les projets innovants (FSPI 2020-2021) : 521 k€ French Embassy in Cambodia, French Ministry for Europe and Foreign Affairs

FSPI Wat-Health 2021-2022: Water and Health Risks in Cambodia

The "Water and Health Risks in Cambodia" project (Wat-Health – 600k€) aims to define the exposure and vulnerability of rural populations to the most serious health hazards related to floods in the Koh Thum district. The study is based on the premise that, as in other countries of the region, water-related hazards such as contaminants, bacterial and vector-borne diseases represent a major potential health threat. Investigations are conducted at a pilot site in the Mekong Delta, located between Phnom Penh and the border with Vietnam. The overall scientific ambition is to determine how changes in river flood regimes affect the distribution of pollutants and lead to changes in the biodiversity of water-related pathogens and disease vectors, with ultimate effects on health, agricultural production and the environment. The MBL is coordinating the ''water-related bacteria'' activities focused on the detection of bacteria (especially Leptospira and Burkholderia) in water and soil samples (surface soil, 30cm, 60cm, 90 cm depth). Monthly field sampling has been performed at 22 sampling sites from July 2021 to September 2022 (15 missions), and these missions allowed us to identify the presence of Leptospira and Burkholderia in Koh Thum. 216/1495 (14.45 %) of the environmental samples tested positive to Leptospira and 561/1443 (38.88 %) tested positive for Burkholderia. Next-generation sequencing of both Leptospira and Burkholderia genomes is ongoing. In parallel, the MBL performed a serological analysis (melioidosis and leptospirosis) on 500 inhabitants of the study area as part of the epidemiological survey conducted by GeoHealth. The seroprevalence of leptospirosis varied between 8.2 % and 13 % using commercial serological tests, and reached 61 % with the reference MAT (Micro Agglutination Technique). Regarding melioidosis, seroprevalence was estimated at 11.58 % using the IHA technique (Indirect Haemagglutination Assay). Global analysis including risk factors and spatiotemporal analysis is ongoing and we will continue exploring the spatiotemporal circulation of Leptospira and Burkholderia at the environment/animal/human interface within the scope of the AFRICAM project. The data on the seroprevalence of leptospirosis was presented at the 12th International Leptospirosis Society Conference 2022 in Bangkok, Thailand by V. Herbreteau (GeoHealth).

Collaborations	PI: S. MASSUEL (G-EAU Unit, IRD; ITC) Team Leaders: M. HIDE and G. DELVALLEZ Medical Biology Laboratory (S. CHENG, S. HAN, S. MENG) MIVEGEC Unit, IRD, LMI DRISA (AL. BANULS) G-EAU (S. MASSUEL, JP VENOT) ESPACE-DEV, IRD (Geo-Health/Epidemiology Unit - V. HERBRETEAU) Entomology Unit (S. BOYER) ITC Institut Pasteur in New Caledonia (C. GOARANT) RUA CNRS
Funding	Fonds de solidarité pour les projets innovants (FSPI 2020-2021): €240,000 for the MBL French Embassy in Cambodia, French Ministry for Europe and Foreign Affairs

Establishing Microbial AMR Genomics in Phnom Penh through Genomic Snapshot Surveys of Multidrug-Resistant Gram-Negative Bacteremia

The rising prevalence of antimicrobial resistance (AMR) represents a threat to public health globally, as emerging AMR mechanisms and multidrug-resistant pathogens compromise the treatment of microbial infections. In Cambodia, the detection of AMR has been traditionally reliant on culture-based antimicrobial susceptibility testing (AST). Even though AST provides direct visual evidence of how a bacterium will interact with an antimicrobial, it generally provides little or no data regarding resistance mechanisms. Whole-genome sequencing (WGS) provides genome-wide information at the single nucleotide level that can be used to identify the presence and mechanisms of AMR, as well as pathogen identity, virulence, and ancestry. Microbial genomics is currently revolutionizing the diagnosis, surveillance and control of AMR. For this reason, this study aims to combine the rapid advances in genomics with public health epidemiology and the best practices in bioinformatics to set up a new paradigm for AMR control in Phnom Penh.

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

In 2022, we conducted a retrospective genomic epidemiology study of an outbreak of fluoroquinolone-resistant *Salmonella enterica* (serovar Paratyphi A) isolated in Phnom Penh, Cambodia. Paratyphoid fever is a severe systemic febrile illness that only affects human hosts and is transmitted via the fecal-oral route. Fluoroquinolones are the first choice for the oral treatment of non-complicated enteric fever. We have also used comparative genomics to investigate an unusual increase in the number of fluoroquinolone-resistant Paratyphi A strains isolated from patients in Phnom Penh, Cambodia in 2020. We sequenced and compared the genomes of these Paratyphi A strains with an international isolate database that represents the breadth of the serovar 2.3 lineage 2.3 diversity. Our comparative genomic analysis revealed that the 2020 outbreak strains descended from a previously described 2013–2015 Cambodian outbreak of lineage 2.3.1 Paratyphi A infections. Our analysis also showed that the Cambodian sub-lineage 2.3.1 remained largely susceptible to fluoroquinolone drugs until 2015 and acquired chromosomal resistance to these drugs during six separate events between late 2012 and 2015. These results are suggestive of a strong selective pressure on the Cambodian Paratyphi A population linked with fluoroquinolone use. Our findings point towards the consequences of uncontrolled and widespread use of fluoroquinolones, which until now, have been the most effective drugs available for the management of enteric fever. A manuscript detailing these results is being prepared and will be submitted for review and publication next year.

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

Circus – Pilot phase in Cambodia

This is a multicentre study aiming to characterize the circulation of Antimicrobial Resistance (AMR) by focusing on Multi-Drug-Resistant (MDR) Enterobacteriaceae and mobile genetic elements between humans, animals and the environment in Burkina Faso, Cambodia, Ivory Coast and Madagascar. This project was built within the framework of the AMR SUD consortium of teams, coordinated by Christian Lienhardt (TRANSVIMI, IRD) and supported by the AVIESAN. In 2022, the project got partially funded to launch its pilot phase in all 4 countries. In Cambodia, the pilot phase aims to test the overall functioning of the project and its feasibility in the local context. The specific objectives are to estimate the prevalence of MDR-E in humans in Cambodia by focusing on the peri-urban habitat and to evaluate the circulation of antibiotic resistance at the human-animal interface and environment in this habitat. The year 2022 was mainly devoted to the preparation for the project's implementation, including the signature of funding/collaboration contracts, the selection of study sites, obtaining support from partners and local authorities, the development of a study protocol, ethical clearance, the purchase of reagents and consumables, and the recruitment and training of staff. The project is planned to start with a kick-off meeting at the beginning of 2023.

Collaborations	PIs: N. GUESSEND (IP in Ivory Coast), A. SALAM, E. CARDINAL (CIRAD), A.L. BAÑULS (IRD) Team Leader for Cambodia: S. CHENG (MBL)
Funding	AVIESAN AMR-SUD ANR, Antibiorésistance : comprendre, innover, agir (AMR): €700,000

Antimicrobial Resistance Circulation along the Mekong and its Delta - ARCIMED

The ARCIMED Project was selected for support in December 2021 for the FSPI's 'OHSEA' ('One Health in Practice in Southeast Asia') call for projects, a funding program led by the IRD that aims to support emerging One Health initiatives. The main objective of this pilot project is to gain insight into the emergence, transmission and dynamics of resistance determinants along the Mekong River in Laos, Cambodia and Vietnam. To do so, we plan to describe the presence and dynamics of AMR determinants over time and correlate it with external parameters such as river hydrology, seasons (dry/wet), the physicochemical properties of water, the presence of other pollutants, and human activity in the vicinity of sampling sites. In 2022, water sampling was conducted in both dry and rainy seasons at 9 sites along the Mekong, Bassac and Tonle Sap rivers in Cambodia. A total of 117 samples were found to contain bacteria (34 % in the dried season and 66 % in the rainy season) linked to waterborne diseases. 40 % of these bacteria were *Aeromonas spp*., 23 % were Enterobacteria and 12 % were *Acinetobacter spp*. The bacteriological analysis, including the phenotypic detection of antibiotic resistance, is still ongoing.

Collaborations	PI: Nguyen Quang Huy (USTH) Team Leaders for Cambodia: S. Cheng (MBL) and C. Pheng (ITC) IRD (MIVEGEC), Institute of Technology of Cambodia (ITC)
Funding	FSPI OHSEA : 22 500€

Prevalence and Risk factors of Human Papillomavirus Infection among Men Who Have Sex with Men and Transgender Women in Phnom Penh, Cambodia

The prevalence of high-risk human papillomavirus (hrHPV) has been previously reported in women in China, but screening for anal HPV infection is rarely performed among men having sex with men (MSM) and transgender women (TGW) in Southeast Asia due to cost and availability of testing. Despite the fact that hrHPV detection is not useful for screening anal lesions, understanding the epidemiology of anal HPV infection is crucial to support and assess the implementation of HPV vaccination programs, particularly in highly exposed populations and low and middle-income countries. The primary objective of this study is to estimate the prevalence of anal hrHPV among asymptomatic MSM and TGW in Phnom Penh, and to identify risk factors associated with hrHPV infection. A cross-sectional study was conducted on self-collected anal swabs from MSM and TGW under the routine STI-screening activities of Men's Health Cambodia, a non-governmental and non-profit organization working on MSM and TGW issues in Cambodia. 162 participants were included, with a median age of 28.1 years. The study found that the prevalence of any HPV-types, hrHPV types and other HPV-type infections were 83%, 66%, and 65%, respectively. Among hrHPV-type infections, HPV16 was the most prevalent (25 %), followed by HPV52 (21 %) and HPV18 (17 %). HPV16, HPV56 and multiple HPC infections were more prevalent in HIV-positive patients. Among hrHPV-positive patients, 50% of infections would be prevented by the 2- or 4-valent vaccines, and 84 % by the 9-valent vaccine, indicating that the introduction of HPV vaccination in this key population would be greatly beneficial. The prevalence of anal infections with NG, CT, and MG was 15 %, 19 %, and 3 %, respectively. Concomitant anal bacterial STIs were identified as a risk factor for hrHPV anal infection. Chlamydia trachomatis infection also tended to be associated with hrHPV infection. Further research in larger and follow-up studies is still required to fully understand how bacterial STIs affect hrHPV infection or persistence. A manuscript detailing these results has been approved and is currently in production.

Collaborations	PIs: Gauthier DELVALLEZ, Sokleaph CHENG and Aude JARY MHC
Funding	IPC, MHC and the ANRS MIE

Pediatric Melioidosis in Cambodia

In order to investigate the epidemiology of melioidosis in Cambodia, we are studying an exhaustive sample of 122 isolates from pediatric meliodosis, collected from June to November 2020 in Kantha Bopha hospitals (Kantha Bopha Hospital [Phnom Penh] and Jayavarman VII Hospital [Siem Reap]) using both a genetic approach, and spatio-temporal analysis. *Burkholderia* species identification has been performed using MALDI-TOF, and the genomes of *B. pseudomallei* isolates have been sequenced by NGS. Both spatial/temporal analysis and genome analysis are ongoing.

Collaborations	Team Leader: M. HIDE Medical Biology Laboratory (K. Vandelanotte, R. Dusadeepong, G. Delvallez, S. Cheng) MIVEGEC Unit, IRD, LMI DRISA (A.L Bañuls) GeoHealth-IRD (V. Herbreteau) Kantha Bopha Children's Hospitals (D. Laurent)
Funding	LMI DRISA

4.6.8 Research Programs – Outlook for 2023

Prezode AFRICAM Cambodia

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

Collaborations	PI: A.L. BAÑULS (IRD) IPC (MBL, Virology, Epidemiology and Public Health Unit, Medical Entomology) IRD (MIVEGEC, Espace-Dev), CIRAD, AVSF (*Agronomes et Vétérinaires Sans Frontières*), IDE (Empowering entrepreneurs to end poverty), WCS (Wildlife Conservation Society), Battambang Hospital, Institute of Technology in Cambodia (ITC), WCS (WILDLIFE CONSERVATION SOCIETY)
Funding	AFD, €1.8 million

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 high-priority public health issue in Cambodia and worldwide. New cervical cancer screening methods are emerging that promise to be superior to the existing Visual Inspection with Acetic Acid (VIA) procedure in Cambodia. 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's main objective is to analyse the effectiveness and cost vs. benefit performance of a modified program for cervical cancer screening assisted by a cloud-based digital system. The IPC's medical biology laboratory will coordinate the project's laboratory activities, which will start in the first half of 2023.

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

ACIP ORACAN "Geographic Origin of the Parasite *Angiostrongylus cantonensis* and the Role of the *Achatina fulica* Mollusc 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* first described in 1935 in China. 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 ingestion of intermediate or paratenic hosts consumed raw or undercooked. Neurologic manifestations include eosinophilic meningitis and encephalitis or encephalomyelitis. Today, human cases of nerve angiostrongylosis are primarily reported in Southeast Asia (Thailand, Malaysia, China), the Pacific Islands, Indian Ocean islands, the Greater Antilles (Cuba, Puerto Rico, Jamaica), and more recently Brazil. Rare cases have also been described in Africa in Egypt and Côte d'Ivoire. The geographic spread of *A. cantonensis* is thought to have been spurred by the dispersal of its hosts, primarily the giant African snail *Achatina fulica*. The first cases of nerve angiostrongyloses in the French Departments of America (FDA) date back to the very end of the 1990s. The main objective of the ORACAN project is to determine the origin of the *Angiostrongylus cantonensis*, a parasitic nematode causing Nerve angiostrongylosis in humans, but also its means of introduction (rats or snails) into the French Departments of America and Africa, about which no data exists. To address these questions, it is mandatory to assess the prevalence and generate phylogenetic data of *A. cantonensis* and of the major intermediate host *A. fulica* in all regions of the world. This project was approved for funding through *Actions Concertées InterPasteuriennes* - ACIP 2022 by the *Institut*

Pasteur and will involve 8 Institutes among the *Institut Pasteur* International Networks in the Africa, Asia-Pacific and America regions. The project will allow us to answer these questions and fill a knowledge gap about of the spread of *A. cantonensis* throughout the world by looking both at *A. cantonensis* and *A. fulica*.

Collaborations	PI: Ferdinand, Séverine (IP in Guadeloupe) MBL: B. Guillard, S. Cheng, S. Heng 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 InterPasteuriennes - ACIP 2022: €49,700

Assessment of Antibiotics in Rice Cultivation Inputs and Risks of Bacterial Resistance Emergence

The use of antibiotics in agriculture is a major concern internationally (WHO, FAO, WHO 2018). Globally as well as in Cambodia, antibiotics are used in agriculture and primarily on rice. This widespread use of antibiotics use is of great concern to the WHO because of the risk of emergence of antibiotic resistance (ABR) in medically-problematic bacteria. Several of these antibiotics (e.g. streptomycin, tetracycline, gentamicin, amoxicillin and oxytetracycline) are considered by WHO as critically important antimicrobials for human medicine. The main objective of this project is to map the use of antibiotics in rice production and to estimate the risk of selection of resistant bacteria. To do this, field surveys will investigate the extent and diversity of antibiotics used to manage rice health problems in Cambodia. In parallel, bacterial diversity and antibiotic resistance mechanisms will be explored in the soil and rhizosphere of various rice fields, and compared with ABR observed in humans through the other MBL projects.

Collaborations	PI: S. BELAFIORE (PHIM Unit, IRD; ITC) MIVEGEC Unit, IRD, LMI DRISA (M. HIDE, A.L. BAÑULS) MBL: S. CHENG ITC (Cambodian PI)
Funding	Under review

4.6.9 Publications List in 2022

NOTE

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

1. **How a PCR Sequencing Strategy Can Bring New Data to Improve the Diagnosis of Ethionamide Resistance.**
   Thomas Maitre, Florence Morel, Florence Brossier, Wladimir Sougakoff, Jéremy Jaffre, Sokleaph Cheng, Nicolas Veziris, Alexandra Aubry and on behalf of the NRC-MyRMA.
   Microorganisms. 2022 Jul 15;10(7):1436.

2. **Non-toxigenic Corynebacterium diphtheriae in hallux ulceration.**
   Gauthier Delvallez, Edgar Badell, Sokleaph Cheng, Soda Meng, Vivandet Tong, Judy Norman, Julie Toubiana, Koen Vandelannoote, Anne-Laure Bañuls, Mallorie Hide, Sylvain Brisse.
   J Infect Dev Ctries. 2022 Jun 30;16(06):1118–21.

3. **Prevalence and factors associated with faecal carriage of extended-spectrum β-lactamase-producing Enterobacterales among peripartum women in the community in Cambodia.**
   Agathe de Lauzanne, Navin Sreng, Elsa Foucaud, Touch Sok, Thida Chon, Chhaily Yem, Veasna Hak, Sothada Heng, Meng Soda, Malika Gouali, Maya Nadimpalli, Malin Inghammar, Mamitina Alain Noah Rabenandrasana, Jean Marc Collard, Muriel Vray, Simon Le Hello, Alexandra Kerleguer, Patrice Piola, Elisabeth Delarocque-Astagneau, Didier Guillemot, Bich Tram Huynh, Laurence Borand, **BIRDY study group**: Andrianirina Zafitsara Zo, Marguerite Diatta, Jean Baptiste Diouf, Fatoumata Diene Sarr, Joseph Faye, Sophie Goyet, Perlinot Herindrainy, Elsa Kermorvant-Duchemin, Siyin Lach, Veronique Ngo, Michael Padget, Feno Manitra Jacob Rakotoarimanana, Bodonirina Tanjona Raheliarivao, Frédérique Randrianirina, Abdoulaye Seck, Arnaud Tarantola, Armya Abdou Youssouf.
   J Antimicrob Chemother. 2022 Sep 30;77(10):2658–66.

4. **Rapid Generation of In-House Serological Assays Is Comparable to Commercial Kits Critical for Early Response to Pandemics: A Case With SARS-CoV-2.**
   Heidi Auerswald, Chanreaksmey Eng, Sokchea Lay, Saraden In, Sokchea Eng, Hoa Thi My Vo, Charya Sith, Sokleaph Cheng, Gauthier Delvallez, Vann Mich, Ngy Meng, Ly Sovann, Kraing Sidonn, Jessica Vanhomwegen, Tineke Cantaert, Philippe Dussart, Veasna Duong, Erik A. Karlsson.
   Front Med. 2022;9:864972.

5. **Mycobacterium tuberculosis genetic diversity and multidrug resistance in Cambodia.**
   S Cheng, M Hidé, SH Pheng, S Chhay, A Kerléguer, G Delvallez, QH Nguyen, TE Mao, TVA Nguyen and AL Bañuls.
   Submitted, oct 2022 Journal of Infection and Drug Resistance

4.6.10 Support to National Authorities

- In partnership with the National Center for HIV/AIDS, Dermatology and Sexually Transmitted Diseases (NCHADS) of Cambodia, follow-up of HIV seropositivity
- Participation in CENAT’s Technical Working Group on Laboratory including the development of technical procedures guidelines
- 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)

4.6.11 Teaching and Training

Continuing Professional Training and Development for the MBL's Staff

Dr Cheng Sokleaph (biologist):

- An introduction to computational biology, IPC (May 2022)
- *Analyse des génomes pour la surveillance de l'antibiorésistance* (IPP, France, June 2022)
- 5th International Masterclass AMR (Online course, Sept 2022)
- The 1st RAPID symposium, "Accelerating the One Health Response on AMR", IPK, Korea (Nov 2022)
- 7th World One Health Congress, Singapore (Nov 2022)
- *Formation Bioinformatique Genesys 2022*, IRD, France (Nov 2022)

Mr Meng Soda (technician, microbiology):
Institut Pasteur de Nouvelle-Calédonie (15 Nov to 02 Sep 2022)

- Training within the framework of Wat-Health project
- Leptospirosis reference serological technique: Microagglutination Technique (or MAT)
- Training for the routine observation and subcultures of a range of Leptospira serovars

Ms Keo Sokunthy (technician, molecular biology):

- Bachelor degree of Medical Laboratory (UHS, 2022-2024)

Ms Khan Ousa (Technician), Mr Saulim Sereyrith (Technician):

- ISO 15189 Medical Laboratory Awareness Training (Punyam Academy, online training)
- Chemical and Biological Risks (Kaptitude, online)

Teaching

Heng Seiha (Engineer, Molecular Biology) and Meng Soda (technician, microbiology): Trainers for the module 6 (Microbiology) in the *Qualifying the Workforce for AMR Surveillance in Africa and Asia* (QWArS) project, in December 2021.

Internships

One of the missions of Institut Pasteur is training, in 2022 we welcomed trainees with various backgrounds:

- Master's Degree, Biology of Infectious Diseases, UHS/Université Paris Saclay: *1 student*
- Master's Degree, Medical Biology, UHS: *2 students*
- BA, Pharmacy, UHS: *12 students*
- Associate's Degree, Medical Laboratory Technician, University of Puthisastra: *6 students*
- BA, Science Research, University of Puthisastra: *4 students*
- High School, Lycée Français René Descartes: *1 student*

4.6.12 Outlook

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 maintaining our standard in terms of technical expertise.

In 2023, we will continue to make evident with our patients and clients that our laboratory is a reference in Cambodia through:

- Our consistency in the quality of care;
- Our use of up-to-date technologies, and procedures and techniques reflecting current best practices;
- Our highly competent staff, who provides services with professionalism.

LIS Change

In 2023, we will change our laboratory information management system (LIS).

DGLab (DATAMED) will replace CODATEC. Start-up scheduled in June 2023.

Implementation of Flow-cytometric Immunophenotyping

In 2023 the collaboration between the MBL, the Immunology Unit of IPC and Kantha Bopha Hospital will continue to bear fruit, with the aim of improving the diagnosis of hematological malignancies in Cambodia. An internal IPC budget (approximately 15,000 dollars) has been allocated to this project.

Development plan

Institut Pasteur du Cambodge must adapt to the evolution of medical biology in Cambodia. In 2023, a development plan for the Medical Biology Laboratory will be implemented.

The different axes of development are:

- Better communication regarding our accreditation and the quality of our services;
- Competency development for the staff (management and polyvalence), for routine and research activities;
- Development of the offered analysis range (tests, analyzers);
- Establishment of service agreements with new external clients;
- Reflection on the evolution of the MBL into a multi-site laboratory.
- This plan involves the Medical Biology Laboratory, the management and the directors of IPC.

Research activities

- To strengthen research activities, at the end of 2022, the MBL's Bacteriology and Antibiotic Resistance (BAAR) Research Group was created with a team of 3 permanent staff (1 researcher and 2 technical staff) within the laboratory. This permanent group is dedicated to research projects, working alongside temporary staff recruited for specific research projects. In 2023, the group will continue to develop their capacities to maintain the research activities at MBL, including the recruitment of local research staff and Master's and Ph.D. students, training activities with new technologies, and building scientific collaboration with national and international teams.
- Increase the output of scientific publications.
- A Bacterial Phylogenomics Group, led by Dr. K. Vandelannoote, will be created and will operate independently of the MBL.

4.7 Laboratory of Environment and Food Safety

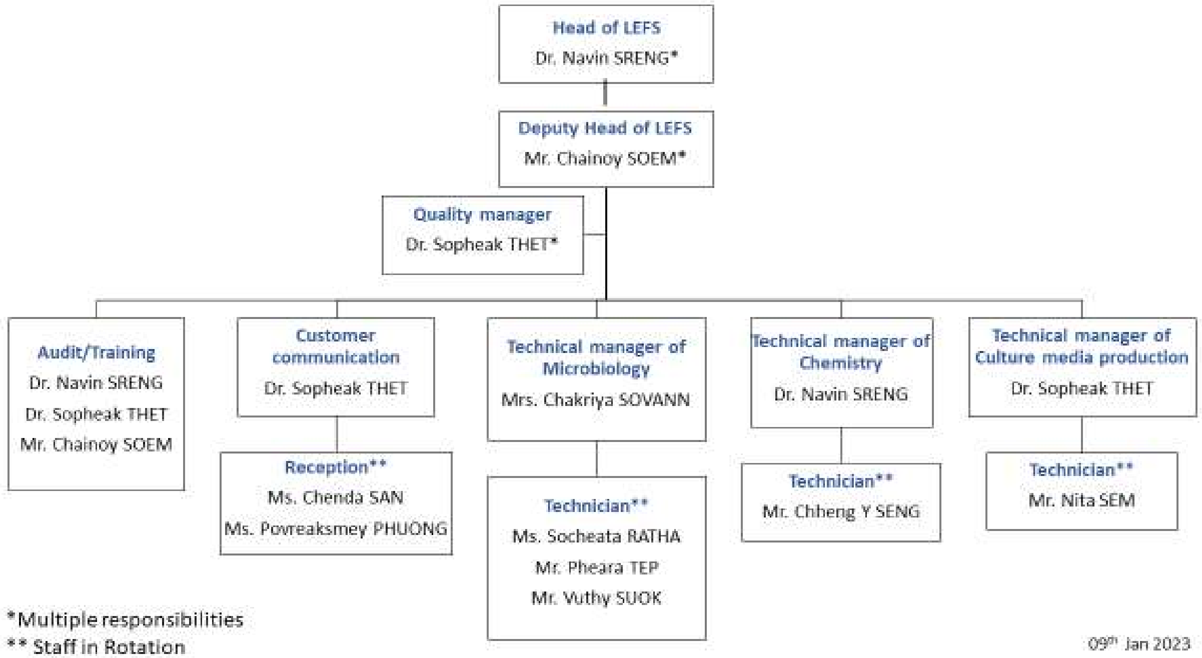
The Laboratory of Environment and Food Safety (LEFS) was created in 1995. Its activities mainly involve microbiological and chemical analyses of food and water. In more detail, the LEFS aims to:

- 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.

4.7.1 Functional Structure



*Multiple responsibilities
** Staff in Rotation

09th Jan 2023

Figure 30. Laboratory of Environment and Food Safety organogram

Changes occurred to the team's composition in 2022: one new technician was recruited to replace a staff member who had left. The Technical Manager in chemistry has also resigned, and recruitment efforts for a replacement are ongoing.

4.7.2 Service Activities in 2022

During 2022, our laboratory tested 8,840 samples, comprising 4,251 food samples, 2,592 water samples for microbiology testing and 1,842 water samples for chemical testing. No training was requested in 2022.

Compared to 2021, the total number of samples tested increased by 33 percent. The year 2021 was difficult one due to the ongoing Covid-19 crisis, whereas the year 2022 saw the end of Covid-19 pandemic, with the tourism and hospitality sectors slowly returning to normal.
The analytical activities (sample numbers and test numbers) over the last five years are shown in the figure 31 below:

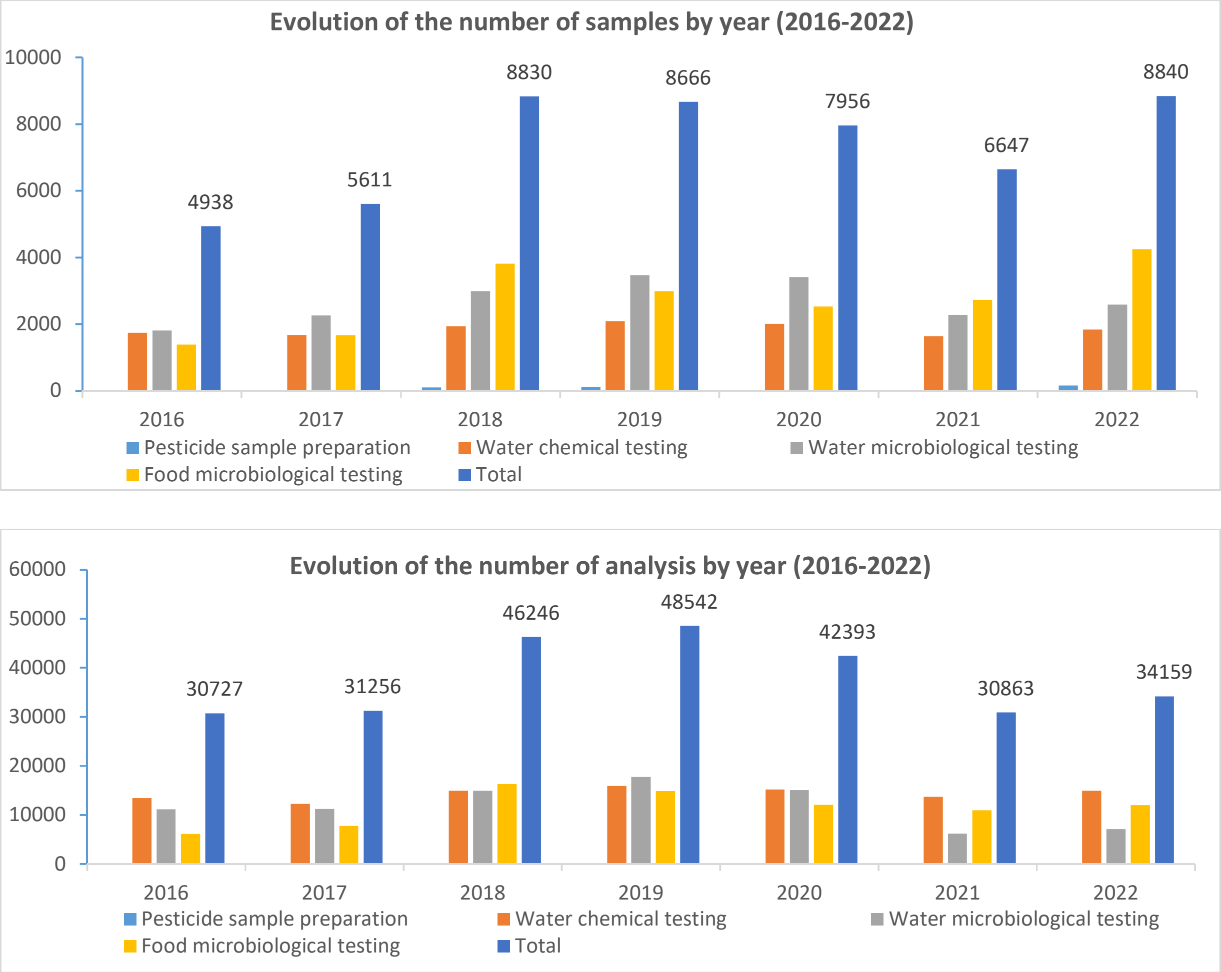


Figure 31. Evolution of the number of samples and analysis by year

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

Food sample

- 45% of food samples (1,925/4,251) were reported to conform to standards/customer requirement although 7% (142/1,925) were unsatisfactory;
- The unsatisfactory results were due to Salmonella contamination (16%), and high levels of hygiene indicators such as Coliforms bacteria (39%), E.coli (9%) and Yeast and Mould (5%). 55% of Salmonella positive food samples were raw meat (13/23) following by 17% of Fish/fishery product (4/23), 17% of Raw ready to eat meal (4/23) and 9% of Pastry products (2/23).

Water

- 23% of water samples for microbiology testing (590/2,592) for which test results were reported to conform to standards/customer requirement, 15% (90/590) were unfit for human consumption because of fecal contamination.
- 28% of ice cube samples (70/250) served in the restaurants and bars were found to be contaminated by fecal bacteria 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 receive the ISO 17025 accreditation, various quality control documents have been created, and their use implemented in the LEFS. Below is the total number of such documents/procedures/forms that are currently in use.

Table 5. Quality Management Documents in LEFS

Documents used in the quality management system	Total number
Quality manual	1
Procedures	83
Forms	133

4.7.3 Research Programs - Major Achievements in 2022

FSPI ARCAHE: Antibiotic Resistance at the Human/Animal/Environment Interface in a "One Health" Approach in Cambodia

Antibiotic resistance (ABR) is an increasing public health concern and threatens to negate decades of infectious disease control efforts. The emergence and spread of ABR are mostly attributed to the overuse of antibiotics, whether among humans, animals, or in the environment. The development of ABR control and monitoring strategies has become a priority in low-middle income countries (LMICs), particularly in Southeast Asia where very high levels of ABR prevalence are reported, as is the case in Cambodia. We observe the emergence of bacteria resistant to multiple antibiotics and having a direct impact on human health. We do not know why and where ABR emerges and how it circulates between humans, animals and the environment in the country. This information is essential for the establishment of effective control measures. Local public health actors in the country, IPC, the *Institut de Recherche pour le Développement* (IRD), CIRAD, and two major hospitals in Cambodia, Calmette (Phnom Penh) and Battambang's provincial hospital (in Cambodia's Northwest), have gathered around this project to improve control of ABR using a "One Health" approach.

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

The **secondary objectives** are:

1. To estimate the prevalence of antibiotic resistance in Calmette Hospital—a key Phnom Penh hospital—and in the Battambang Provincial Referral Hospital, considering the elevated number of community infections in that province.
2. To study the circulation of resistant bacteria in the environment (animals, soil, food, water) of patients with resistant bacteria who were admitted at Battambang's hospital.
3. To contribute to the development of research capacities and scientific skills on ABR and the "One Health" concept.

LEFS's responsibility in the project is part of the secondary objective no. 2 (SO2). It involves working on food and environmental (soil, water, and wastewater) samples collected from the surroundings of selected patients. LEFS's activities within this project has ended in 2021, however, in-depth genetic studies are in progress.

Table 6. Bacteria detected and their resistance phenotypes in food and environmental samples (data in 2021)

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

FQ-Rs: Resistant to fluoroquinolones

Hcase: High-level cephalosporinase

CPE: Carbapenemase

MRSA: Methicillin-resistant *staphylococcus aureus*.

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

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

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

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

The **main objective** 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:
   a. Identify two prominent bacterial pathogens associated with vegetable-borne disease(s);
   b. Conduct a longitudinal study to map and characterize microbial pathogen contamination points, persistence, and transmission in vegetable supply chains;
   c. Use previously collected data to create a shared research agenda among all partners in terms of critical control points to be targeted for high-impact interventions.
2. Creation of targeted Interventions:
   a. Identify and/or design interventions to reduce microbial contamination;
   b. Assess food safety awareness and willingness to apply potential interventions for specific control points;
   c. 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:
   a. Create and deliver engagement programs to foster greater adoption of food safety measures by farmers, distributors, vendors, and market management groups;
   b. Deliver engagement programs that improve food safety awareness among consumers;
   c. 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.

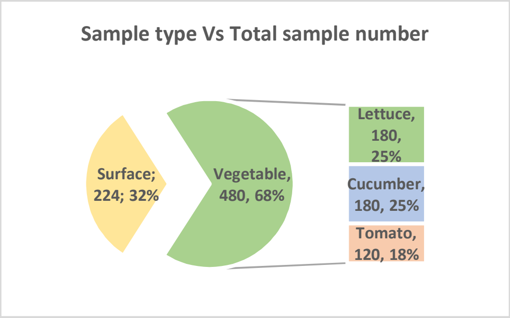
30 farms, 30 distribution centre vendors and 30 market vendors were included in this project as sampling sites for each province. Cucumbers, lettuce, and tomatoes were defined as high-risk vegetables (if eaten raw) and thus selected for this project. Besides vegetables, contact and non-contact surface 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:

Table 7: Sample number according to location

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



704 samples were collected from Siem Reap Province: 224 samples from farms, 240 samples from distribution centre vendors, and 240 samples from market vendors. 68 % of these samples were vegetables (25 % lettuce, 25 % cucumbers, 18 % tomatoes), and 32 % were surface samples.

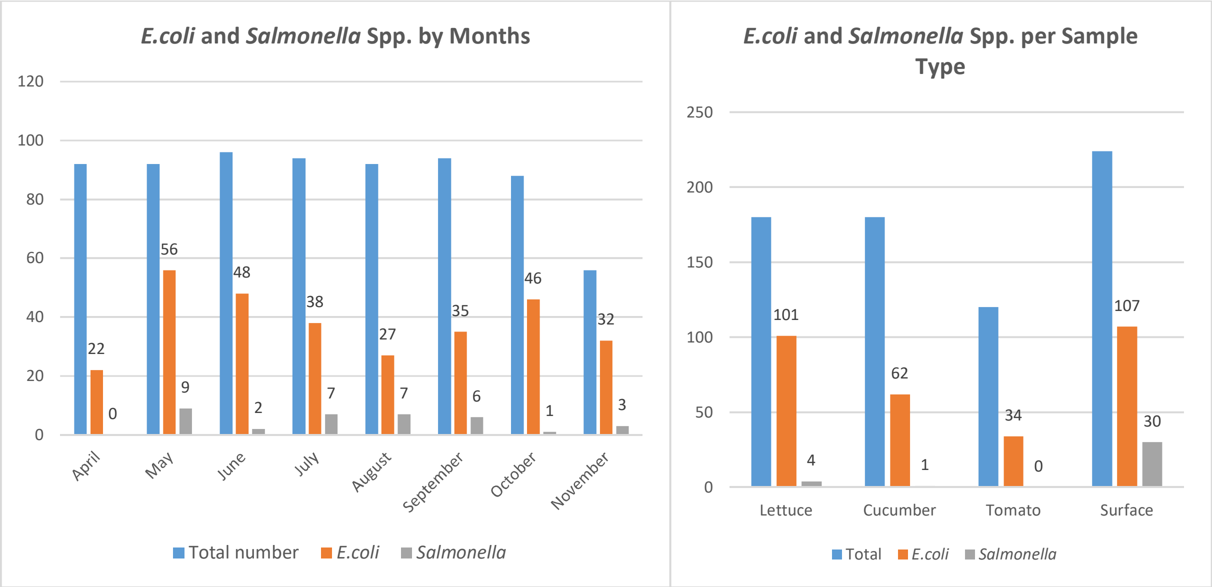


Figure 32. E. coli and Salmonella spp. contamination per month and sample type

Within the eight months (missions) of sample collection, the *E. coli* contamination rate was found to be highest in May, October and November. *Salmonella* contamination was low compared to *E. coli,* and the highest contamination ratio (about 10%) was found in May.

Regarding the sample types, *E. coli* was more likely to be found on lettuce and surface samples, whereas *Salmonella* was mainly found in surface samples. None of the tomato samples were contaminated with *Salmonella*.
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).
Upon reception of this WGS data, a relevant intervention will be designed and applied for stakeholders (farmers, distribution centre vendors and market vendors) in years 3 and 4 of the project.

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)

4.7.4 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 2022, as part of a national monitoring program, the Ministry of Health sent 862 samples to the LEFS. These samples were obtained through sampling campaigns of industrial foods imported from various countries.

4.7.5 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.

Table 8. Internship Students at the LEFS in 2022

University	Number of students	Program Year	Period (Month)	Date
Royal University of Phnom Penh	2	Year 4	3	07/02/2022–24/06/2022
University of Health Sciences (UHS)	5	Year 4	1	03/01/2022–07/02/2022 14/02/2022–29/03/2022 16/05/2022–05/07/2022
Institute of Technology of Cambodia	2	Year 3	2.5	15/07/2022–30/09/2022
University of Puthisastra	2	Year 2	0.5	15/09/2022–30/09/2022 03/10/2022–15/10/2022

4.7.6 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 and water microbiological testing;
- Set up state-of-the-art testing equipment and procedures for chemical analysis in water, using AAS/ICP-OES/MP-AES;
- Set up pesticide, heavy metals, and antibiotic residue analysis capabilities;
- Develop increasing cooperation with internal and external partners for research projects.

4.8 Vaccination Service

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

4.8.1 Functional Structure

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

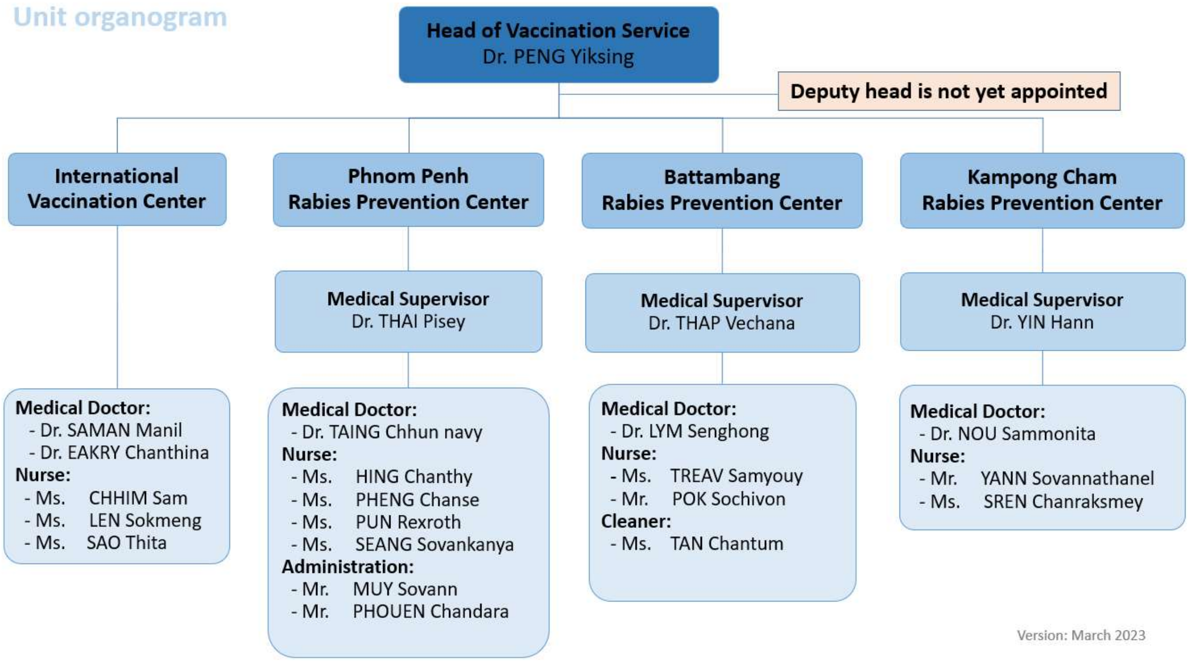


Figure 33. Vaccination Service Organogram

4.8.2 Rabies Prevention Centers

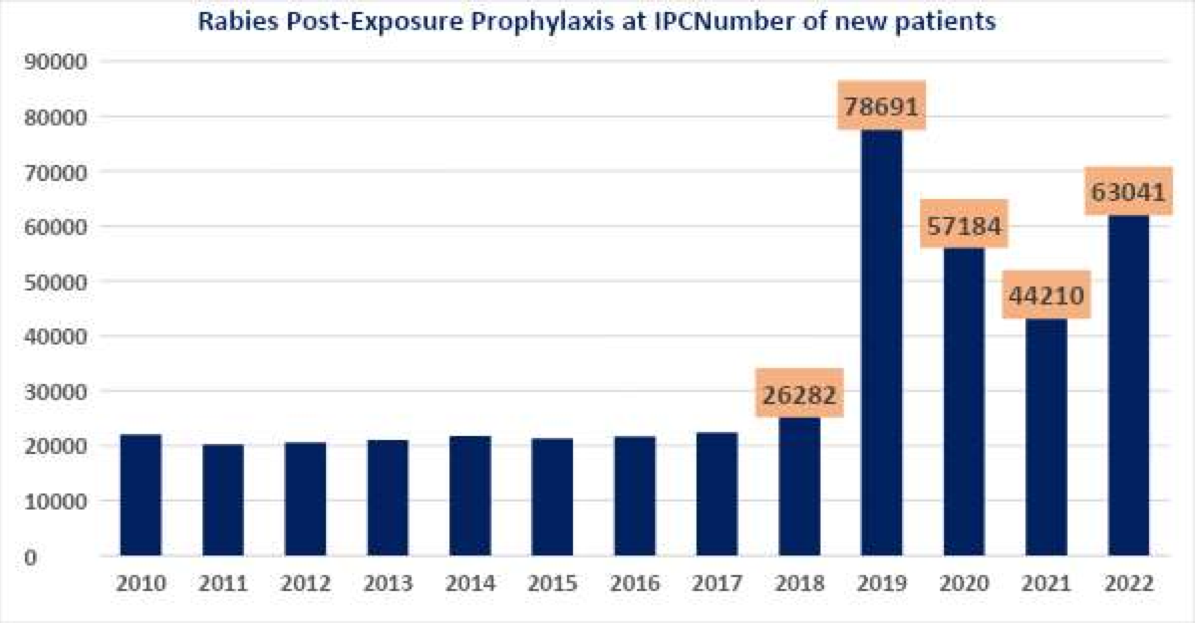
The three rabies prevention centers employ 17 full-time staff under the leadership of the Head of the Vaccination Service. These centers provide post-exposure prophylaxis (PEP) against rabies, including the administration of Equine Rabies Immunoglobulins (ERIG) at an affordable price to the public, as the treatment is subsidized by the Institut Pasteur du Cambodge.

Since July 2018 and following the 2018 WHO recommendations, IPC offers 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 offered 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.

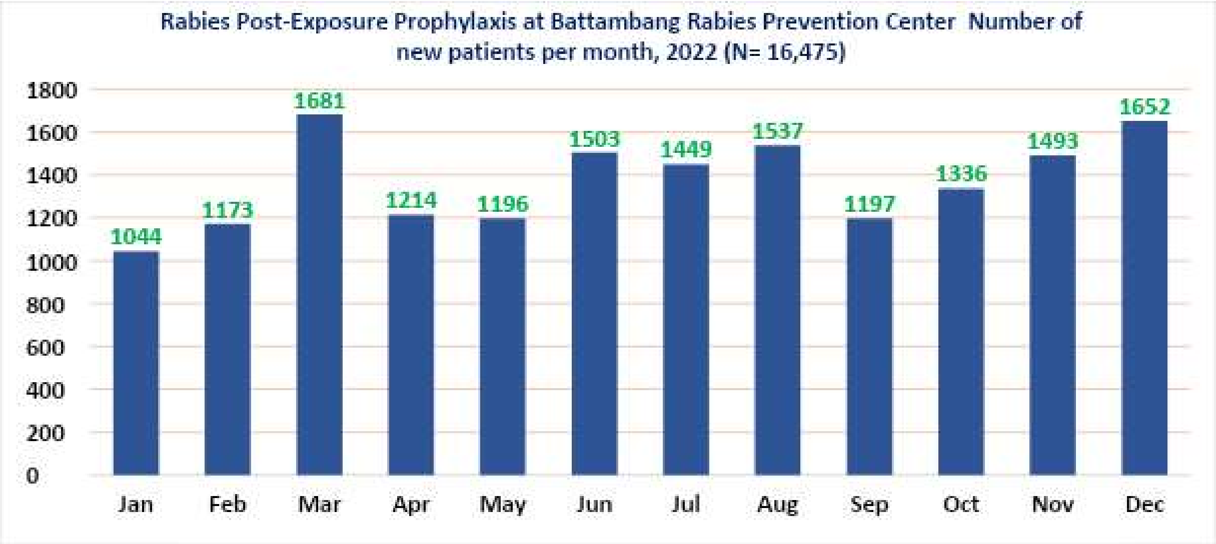
Rabies Prevention Centers Activities in 2022:

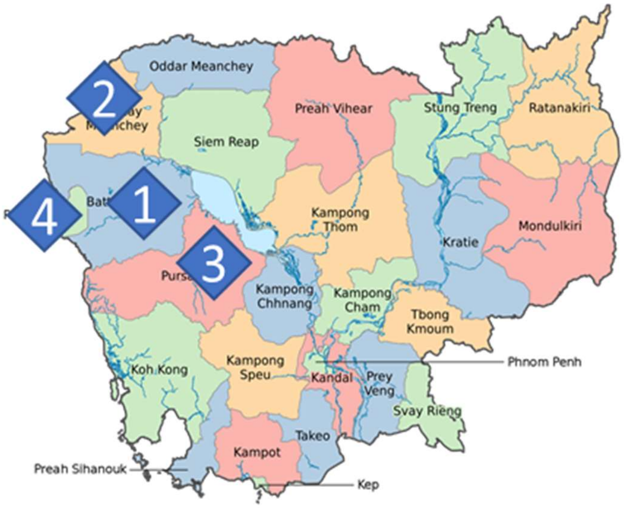
- Provided rabies post-exposure prophylaxis to 63,041 patients.
    - 37,233 patients received rabies PEP at the Rabies Prevention Center in Phnom Penh
    - 16,475 patients received rabies PEP at the Rabies Prevention Center in Battambang
    - 9,333 patients received rabies PEP at the Rabies Prevention Center in Kampong Cham
- **RPC's reach increased by 43% compared to 2021**. The activity levels rebounded quickly after the COVID-19 situation in Cambodia significantly improved.
- A total of 238 animal heads were tested by immunofluorescence for rabies virus at the Virology Unit. 175 samples (74%) were positive for rabies:
    - 78% of all dog samples (167/214) were positive for rabies,
    - 32% of all cat samples (7/22) were positive for rabies, and
    - 1 sample of a symptomatic cow was positive for rabies as well.
- The animal rabies diagnosis information is regularly communicated to MoH, CDC, WHO, and GDAPH.



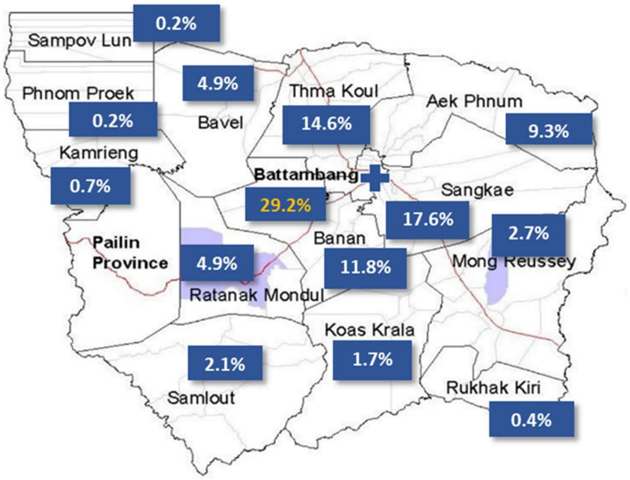
4.8.2.1 Battambang Rabies Prevention Center

The Center located within the provincial hospital was opened in July 2018, following a memorandum of understanding signed on 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.


Rabies Post-Exposure Prophylaxis at Battambang Rabies Prevention Center Number of new patients per month, 2022 (N= 16,475)



Provinces	**%**
1. Battambang	**90.3 %**
2. Banteay Meanchey	6.7 %
3. Pursat	1.5 %
4. Pailin	0.7 %
Other	0.8 %

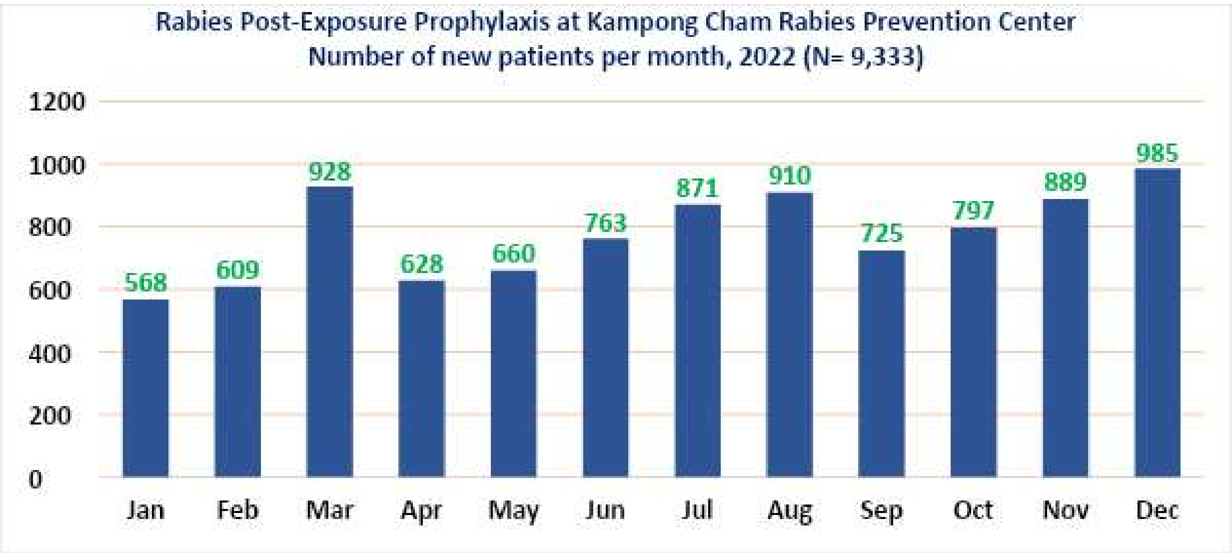


Origin of patients who reside within Battambang province (N=14,879)

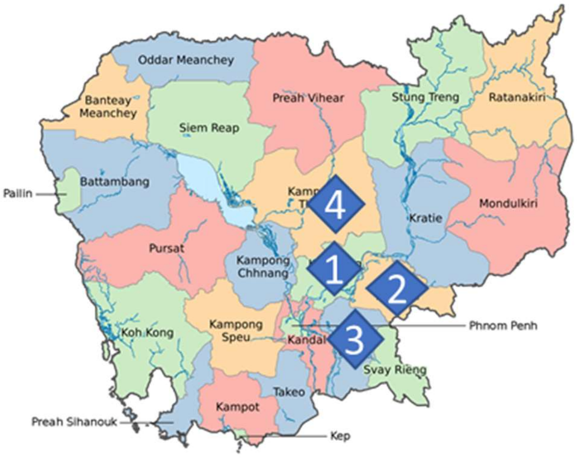
✚ **Battambang Rabies Prevention Center**

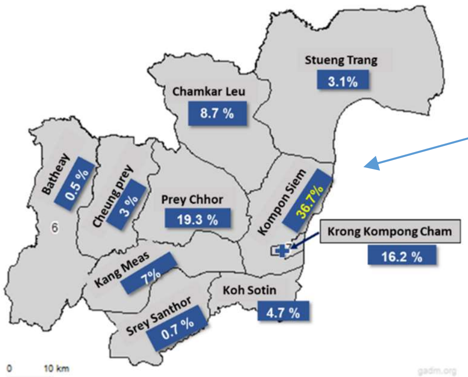
4.8.2.2 Kampong Cham Rabies Prevention Center

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. A draft agreement between IPC and Kampong Cham PHD regarding the construction of a permanent rabies prevention center within the Kampong Cham Provincial Hospital is being reviewed by all parties. This permanent center is expected to be built in the second trimester of 2023 as part of the ongoing collaboration between IPC and the Kampong Cham PHD.



Provinces	%
1. Kampong Cham	**76,2 %**
2. Tboung Khmom	19,9 %
3. Prey Veng	1,6 %
4. Kampong Thom	1,1 %
Other	1,2 %





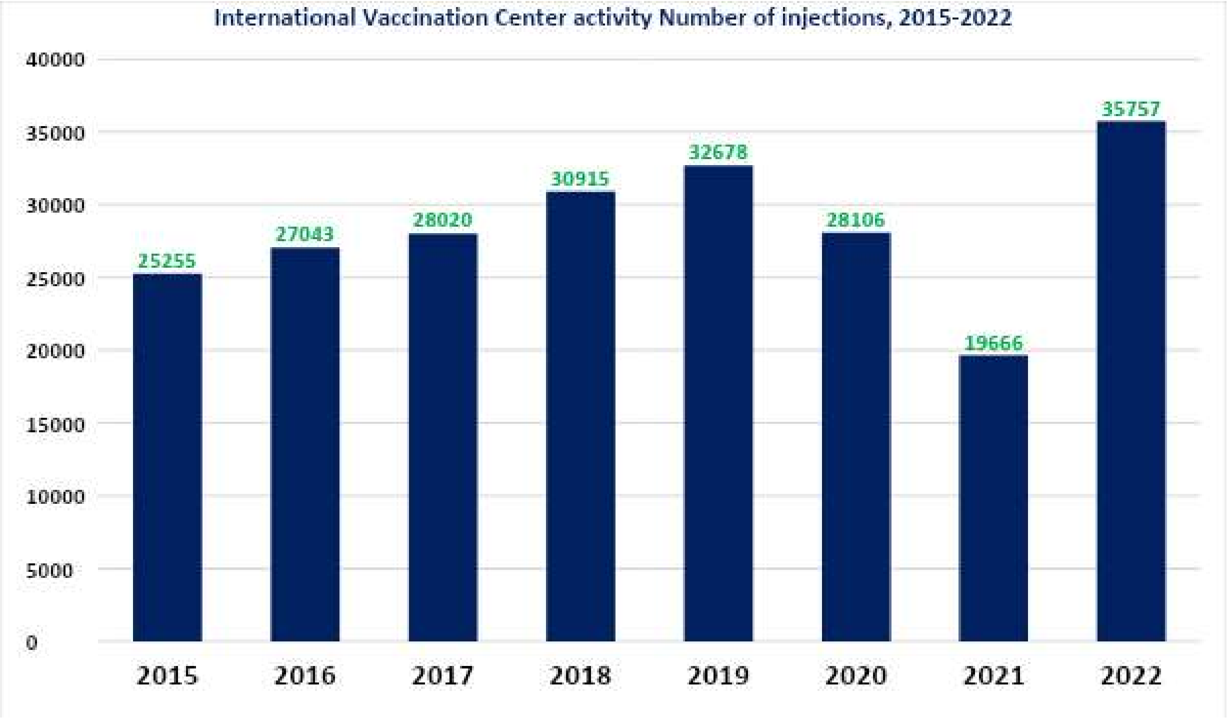
Origin of Patients who reside within
Kampong Cham Province
(N=7,111)

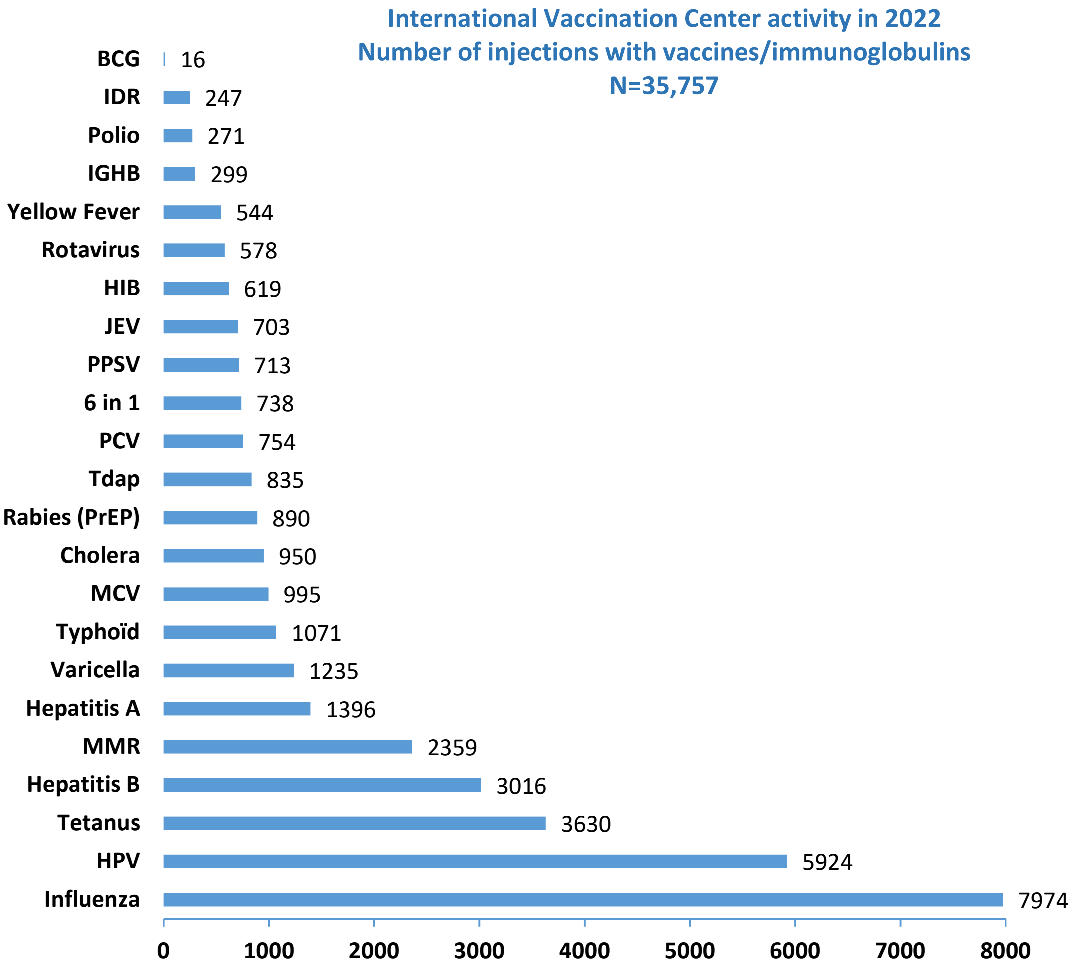
✚ **Kampong Cham Rabies Prevention Center**

4.8.3 International Vaccination Center

The International Vaccination Center at the Institut Pasteur du Cambodge has a medical team of 4 full-time staff under the responsibility of the head of the Vaccination Service. One additional nurse will be recruited in 2023 to support the team in response to the significant increase in the number of patients. 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 2022, a total of 35,757 injections (including the immunoglobulins) were done as part of 22,052 vaccine protocols. IVC's activity has significantly increased, by 82 % compared to 2021. The activity level quickly recovered following the adaptation of our strategic plan to address vaccine shortage issues, and the significant improvement of the COVID-19 situation in Cambodia.





4.8.4 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, and provides rabies PEP to the public at an affordable price.

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

4.8.5 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 services

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

4.8.6 Publications List in 2022

NOTE

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

1. **Accessibility to rabies centers and human rabies post-exposure prophylaxis rates in Cambodia: A Bayesian spatio-temporal analysis to identify optimal locations for future centers.**
   Jerome N. Baron, Véronique Chevalier, Sowath Ly, Veasna Duong, Philippe Dussart, Didier Fontenille, Yik Sing Peng, Beatriz Martínez-López.
   PLoS Negl Trop Dis. 2022;16(6):e0010494. DOI: 10.1371/journal.pntd.0010494

2. **Impact of the switch from four to three intradermal rabies post-exposure prophylaxis sessions in patients bitten by dogs: A cost-consequence analysis from the patients’ perspective.**
   Alicia Le Bras, Kevin Zarca, Yiksing Peng, Malen Chan, Isabelle Durand-Zaleski.
   One Health. 2022;15:100408. DOI: 10.1016/j.onehlt.2022.100408

4.9 Technical platforms

4.9.1 Biobank

4.9.1.1 Background

IPC Biobanking was initiated in 2015 by an internship student in Management of Biobanking (Ms. Sara Cashillo). In 2017, the development of the biobank's software was undertaken by Mr. Stephane Grenier and Ms. Lim Pisey along with a local developer, DEV KHMER S.A.R.L. In 2018, the biobank's software was fully developed and was launched for use, under the care of Ms. Lim Pisey as Biobank Manager. In 2019 and 2020, users encountered a problem with the export function. After this issue was fixed by DEV, users continued to encounter similar issues. In 2020, the biobank's software was re-developed, and it has been used since July 2022.

4.9.1.2 Functional Structure

The biobank section is under the authority of the HSeQM service/Administration unit. It has a cross-cutting role that supports the research units and testing laboratories of IPC with various materials, including documents such as standard operating procedures (SOPs), and software to monitor the samples. Its key responsibilities and functions are as described below.

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

Current users of the biobank are the Virology, the Epidemiology and the Public Health Units, in addition to the Laboratory of Environment and Food Safety.

All samples are currently stored at -80°C in freezers, of which we now have 43 units. In the future, some samples will be stored in liquid nitrogen (N_2); IPC has its own liquid nitrogen generator for daily production.

All these freezers are installed in a purpose-designed room with air conditioners working permanently. This room is only accessed by authorized persons, and entry is strictly controlled. The freezers are constantly monitored through the Oceasoft system, whose use is dedicated to this purpose alone.

4.9.1.3 Biobank Database (11 Projects)

There are currently 11 research projects, with approximately 32,407 samples stored in the databases as per the detail in table below:

Collection/Project	**No. of samples**	**Samples type**	**Storage**
LEFS			
Food Safety Innovation Lab	339	*E. coli* *Salmonella*	Freezer -80°C (HSM-04) Freezer code: I/01599
LBM			
Aspergilloma in Cambodia	684	Serum Aspergillus	Freezer -80°C (HSM-04) Freezer code: 439
VIROLOGY			
ECOMORE 2	2296	Serum Saliva	Freezer -80°C (HSM-04) Freezer code:
Immuno PEP follow up 2019	116	Serum	Freezer -80°C (VIR-02) Freezer code: 1324
RAB00056 IM/ID (Sanofi study)	12	Serum	Freezer -80°C (VIR-02) Freezer code: 1324
Rabies Surveillance	4749	Ammon's horn Spinal bulb	Freezer -80°C (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 Humans in Cambodia	1,940	Kidneys, Swab, Urine, Heart, Lung, Liver, Ectoparasite, Blood clot, Blood serum, Pool organ	NA
Clinical research			
Lowering Interleukin-1 Receptor Antagonist Concentrations after TB Treatment Onset: A Proof of Concept Study in Cambodia and Ivory Coast	237	Plasma Buffy coat	Freezer -80°C (HSM-04) Freezer code: LILAC-TB
TB-Speed Output 2 - Severe pneumonia	1,116	Plasma Stool Whole blood	Freezer -80°C (HSM-04) Freezer code: 1551
Tenofovir as Prevention of Hepatitis B Mother-to-Child Transmission	20,914	Plasma Blood Red blood cell Buffy coat	Freezer -80°C (HSM-04) Freezer code: 1475
Zika Sentinel Surveillance in Prenatal Care Visit and Maternity Ward in Calmette Hospital (Phnom Penh, Cambodia)	4	Serum Urine	Freezer -80°C (HSM-04) Freezer code: 439
Total samples	**32,407**		

4.9.1.4 Action Plan

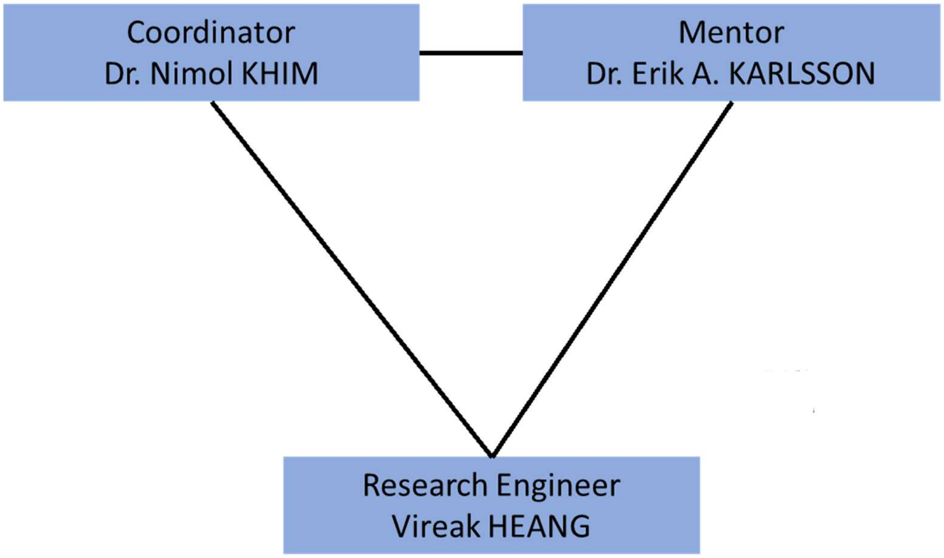
The biobank's activities should be centralized under the responsibility of one (01) person who would take care of the inputs and outputs of samples. The SOPs for the management of a biobank are being written.

4.9.2 Sequencing Mini-Platform

4.9.2.1 Functional Structure

Institut Pasteur du Cambodge established a mini-platform for sequencing in April 2021, with support from a number of stakeholders, including the Agence Française de Développement (AFD), MediLabSecure, the British Embassy in Cambodia, the Wellcome Trust, and the World Health Organization. This mini-platform is intended to strengthen the operational research and public health response at IPC, and is composed of one Principal Investigator in charge of coordination (20 % of work hours, Khim Nimol, Deputy Head of the Malaria Research Unit), one Mentor PI (10 % of work hours, Erik A. Karlsson, Deputy Head of the Virology Unit), one research engineer and one laboratory technician (until February 2022). The laboratory, whose creation was the project's initial step, is also coordinated by other researchers within the institution, such as Cécile Troupin (Virology Unit) and Koen Vandelannoote (Medical Biology Laboratory).

ORGANOGRAM OF THE SEQUENCING MINI-PLATFORM IN 2022



4.9.2.2 Research Programs – Major Achievements in 2022

Our activities, which were focused on amplicon-based and metagenomic sequencing, include wet lab procedures, general raw data clean-up, as well as project guidance and integration for all IPC researchers. A consultation has been developed to help any scientists or collaborations to strengthen and sustain our laboratory capabilities.

So far, our sequencer (a MiSeq system) has been used by two research-oriented entities within internal collaboration projects:

- In partnership with the Malaria Research Unit, the **amplicon deep sequencings of five molecular markers (*ama1-D3*, *cpp*, *cpmp*, *csp* and *msp7)* from 150 *Plasmodium falciparum*** were produced with our MiSeq system in 2022. New wet lab assays were performed by technicians from the Malaria Research Unit and supervised by our research engineer. Libraries were pooled at approximately equal concentration and sequenced on this system to generate 600-bp paired-end readings. All of the raw data (.fastq files) was transmitted to the Principal Investigator (Malaria Research Unit) for further bioinformatics analysis. This project investigated the utility and benefits of amplicon deep sequencing for genotyping of antimalarial drug efficacy in *Plasmodium falciparum* infection.

Collaborations	Malaria Research Unit, Sequencing Mini-Platform, IPC
Funding	World Health Organization, IPC internal collaboration projects

- A research proposal entitled **"In the Air Tonight: Metagenomic Pathogen Discovery as Tools in Pathogen Surveillance**" was submitted to the Centers for Research in Emerging Infectious Diseases (CREID). This project, led by the mini-Platform research engineer (Mr Heng Vireak) and a postdoctoral researcher in the virology unit (Dr Jurre SIEGERS), was successfully funded by EID-SEARCH through a one-year pilot-project grant starting in June 2022, with strong support from our Mentor PI, Dr E. Karlsson. A wet-bench lab has been designed by our research engineer himself. The Illumina MiSeq Reagent Kit v3 150 cycles has been used to obtain sequenced paired-end readings. The generated raw data was uploaded to Genome Detective for data analysis. This funded pilot project greatly increases capacity and capabilities in metagenomic viral discovery, and increases the platforms' visibility within high-profile Next Generation Sequencing (NGS) networks, especially with its innovative engineering.

Collaborations	Virology Unit, Sequencing Mini-Platform, IPC
Funding	NIH Grant, CREID EID-SEARCH Research Center

- In collaboration with the Virology Unit, the MiSeq system has been also used for a study protocol entitled **"Human papillomavirus E6 and E7 coding gene variations and their possible association with the occurrence of cervical intraepithelial neoplasia**". The manipulations, using Illumina MiSeq Reagent Kit v3 600 cycles, were done by technicians from the Virology Unit, supervised by our research engineer. The generated raw data (.fastq files) was sent to the Principal Investigator (Virology Unit) for further bioinformatic analysis.

Collaborations	Virology Unit, Sequencing Mini-Platform, IPC
Funding	IPC Internal collaboration projects

4.9.2.3 Research Programs – Outlook for 2023

- Our research engineer is continuing to conduct a bench wet lab as part of the study named “In the Air Tonight: Metagenomic Pathogen Discovery as Tools in Pathogen Surveillance”. The generated raw data will continue to be uploaded to Genome Detective for data analysis.

- In collaboration with the Malaria Research Unit, this sequencer will be used for targeted deep amplicon sequencing of antimalarial resistance markers *(Pfk13, Pfcrt, Pfmdr1, Pfcytb)* in *Plasmodium falciparum*. This 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 Greater Mekong Subregion.

- Discussions and project plans are also in progress about using this sequencer for studying the circulation of Salmonella serovars in food in Cambodia, to seek internal collaboration within IPC (Sequencing Mini-Platform, Virology Unit, Laboratory for Environment and Food Safety, Bacterial Phylogenomics Group).

- Discussions and project plans developed by the Virology Unit are also in progress regarding the use of the Mini-Platform/sequencer for studying coronaviruses. Furthermore, grant submissions are being prepared for the study of Major Histocompatibility Complex genes, which would help assess environmental surveillance techniques for detecting infectious disease emergences or outbreaks in live animal markets in Cambodia.

4.9.2.4 Outlook (Two to Five years)

This mini-platform was established in April 2021. So far, it has been used by two research entities within IPC. More effort is needed to convince IPC’s PIs to use this platform to increase sequencing capacity. For prospective projects and grants, IPC’s PIs will need to be encouraged and inspired to fully utilize and fund the platform instead of shipping samples to other institutions and partners. Furthermore, genomic surveillance and NGS are increasingly used in many public health initiatives, making it essential to maintain this sequencing mini-platform for the ongoing struggle against the current pandemic, and to prevent the next one.

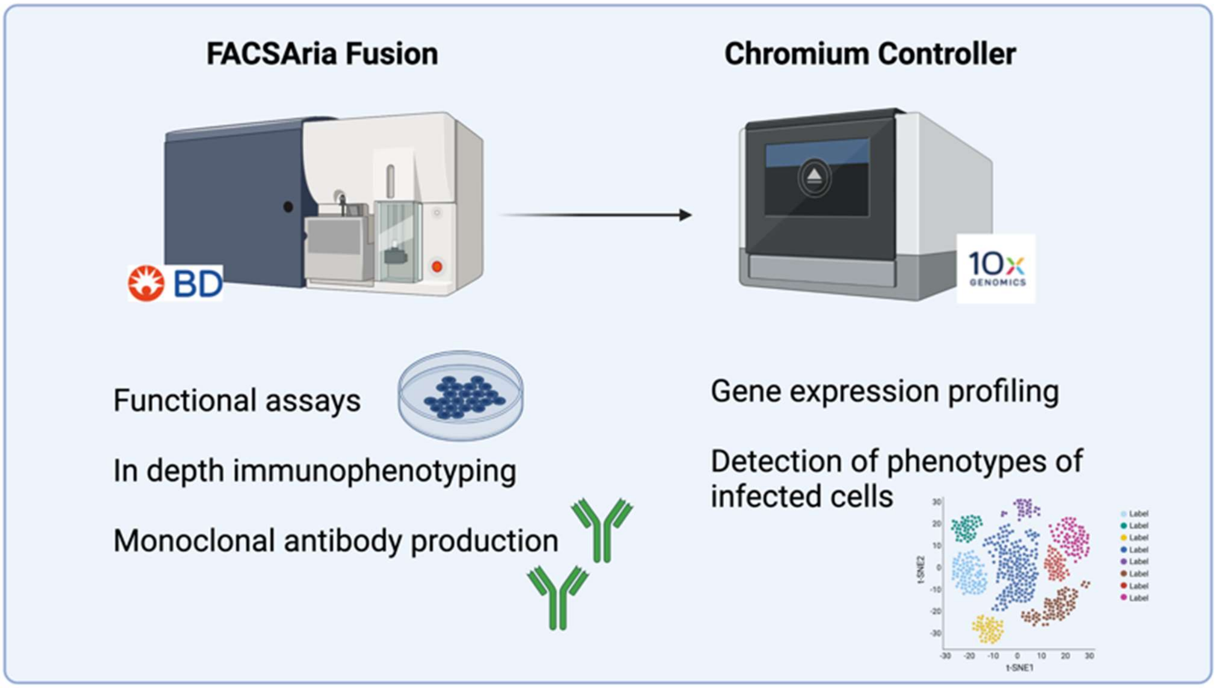
In perspective, training for writing better grant proposals would be required, with support from the Mentor PI. Stronger encouragement from IPC’s Management to the IPC PIs to fully perform NGS analyses with this platform will be recommended. Support from Bioinformatics and IT is critical for data analysis. Involving the BioInformatics Working Group, which was created in 2022 within IPC, would allow us to cooperate with other scientists to strengthen capacity building in Cambodia and beyond.

4.9.3 Single Cell Analysis Platforms

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 at single-cell level directly on site in a low/middle income country. The availability of this equipment and of patient cohorts in the same location allows us to advance our basic research on infectious diseases in Cambodia, which is of major importance.

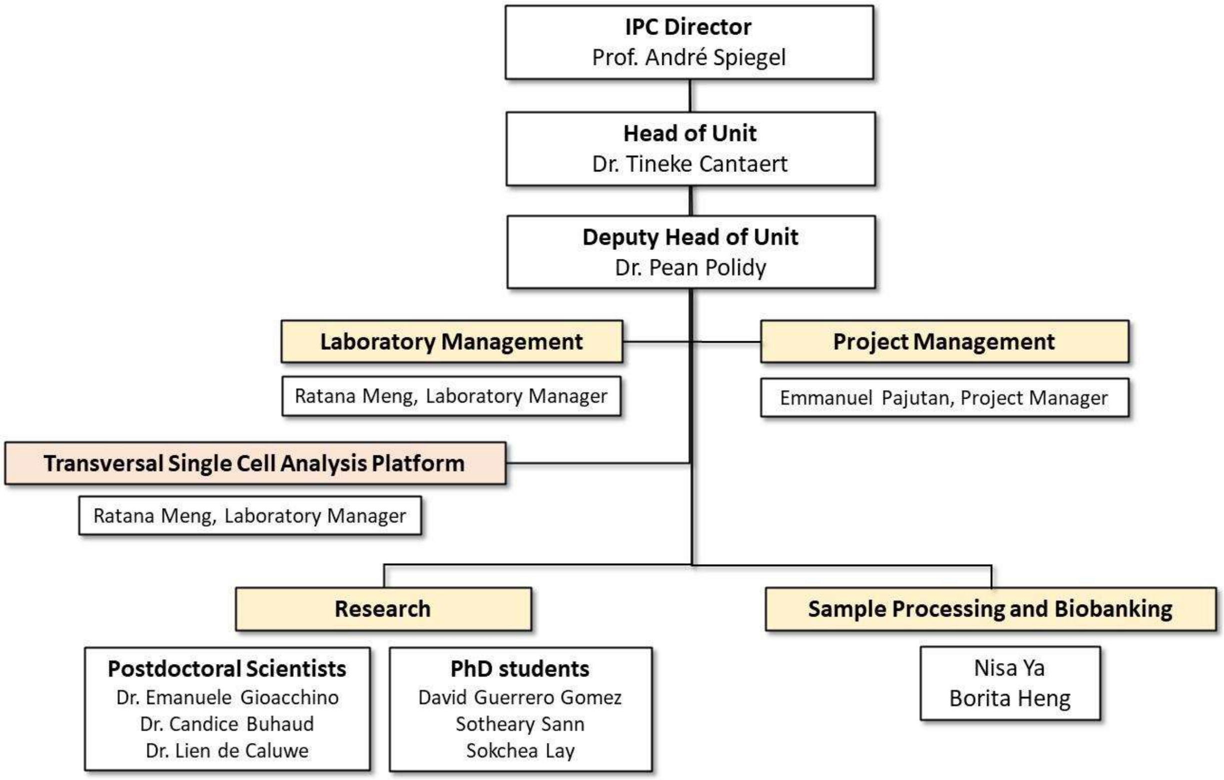
In 2020, we purchased and implemented a new 4-laser, 18-color single-cell sorter (FACSAria Fusion III). Funding was obtained from the Wellcome Trust (Multi-User Equipment grant, PI: Cantaert TINEKE, Co-investigators: Jean Popovici, Benoit Witkowski and Erik Karlsson) and from GIZ. Moreover, we purchased and implemented a 10x Genomics Chromium Controller for single-cell RNA sequencing, funded by the NIH PICREID grant (country PI: Tineke Cantaert). Both pieces of equipment are placed in a BSLII+ biosafety environment.

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 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 their experiments.



4.9.3.1 Functional structure of the platform

The platform is embedded within the Immunology Unit.



4.9.3.2 Research programs – major achievements in 2022

In 2022, the platform was used for the following research projects:

- Understanding the role of regulatory T cells in dengue virus infection;
- Evaluation of the skin's immune response to mosquito saliva (Guerrero, Vo et al., Nat Comm 2022) in a collaborative project with the NIH (Dr Jessica Manning);
- Understanding the mechanisms of Plasmodium vivax receptor-ligand interactions involved in reticulocyte invasion

4.9.3.3 Research programs – prospects 2023

- Assessment of a protective immune response to *Plasmodium vivax*;
- Mechanistic understanding of the involvement of the T cell response in the development of chronic chikungunya disease;
- Generation and analysis of monoclonal antibodies (e.g., dengue, COVID-19…);

4.9.3.4 Perspectives

The platform can be used to study interactions between hosts and pathogens of major health importance in Cambodia. The platform can help in the design, planning and execution of the experiments. In 2023, we plan to hire a bioinformatician who would help with the single-cell RNA-sequencing data analysis and could be involved in various collaborative projects. 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), attached to the 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 will be held in September 2023 open to Cambodian students and other students from low-middle income countries. It will improve the IPC's international visibility and attract foreign scientists. In addition, we continue to train internship and thesis students from various universities in these state-of-the-art techniques.

5. Scientific Publications in 2022

NOTE

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

1. **A Look inside the Replication Dynamics of SARS-CoV-2 in Blyth's Horseshoe Bat (Rhinolophus lepidus) Kidney Cells**
   Heidi Auerswald, Dolyce H. W. Low, Jurre Y. Siegers, Teyputita Ou, Sonita Kol, Saraden In, Martin Linster, Yvonne C. F. Su, Ian H. Mendenhall, Veasna Duong, Gavin J. D. Smith, Erik A. Karlsson.
   Microbiology Spectrum. American Society for Microbiology 0(0):e00449-22. DOI: 10.1128/spectrum.00449-22

2. **A Novel AICDA Splice-Site Mutation in Two Siblings with HIGM2 Permits Somatic Hypermutation but Abrogates Mutational Targeting**
   Johannes Dirks, Gabriele Haase, Tineke Cantaert, Lea Frey, Moritz Klaas, Christian H. Rickert, Hermann Girschick, Eric Meffre, Henner Morbach.
   J Clin Immunol. 2022;42(4):771-82. DOI: 10.1007/s10875-022-01233-5

3. **Accessibility to rabies centers and human rabies post-exposure prophylaxis rates in Cambodia: A Bayesian spatio-temporal analysis to identify optimal locations for future centers**
   Jérome N. Baron, Véronique Chevalier, Sowath Ly, Veasna Duong, Philippe Dussart, Didier Fontenille, Yik Sing Peng, Beatriz Martínez-López.
   PLoS Negl Trop Dis. 2022;16(6):e0010494. DOI: 10.1371/journal.pntd.0010494

4. **Aerosol exposure of live bird market workers to viable influenza A/H5N1 and A/H9N2 viruses, Cambodia**
   Paul F. Horwood, Srey Viseth Horm, Sokhoun Yann, Songha Tok, Malen Chan, Annika Suttie, Phalla Y, Sareth Rith, Jurre Y. Siegers, Sorn San, Holl Davun, Sothyra Tum, Sowath Ly, Arnaud Tarantola, Philippe Dussart, Erik A. Karlsson.
   Zoonoses and Public Health. n/a(n/a). DOI: 10.1111/zph.13009

5. **Antibodies as drugs-a Keystone Symposia report**
   Jennifer Cable, Erica Ollmann Saphire, Adrian C. Hayday, Timothy D. Wiltshire, Jarrod J. Mousa, David P. Humphreys, Esther C. W. Breij, Pierre Bruhns, Matteo Broketa, Genta Furuya, Blake M. Hauser, Matthieu Mahévas, Andrea Carfi, Tineke Cantaert, Peter D. Kwong, Prabhanshu Tripathi, Jonathan H. Davis, Neil Brewis, Bruce A. Keyt, Felix L. Fennemann, Vincent Dussupt, Arvind Sivasubramanian, Philip M. Kim, Reda Rawi, Eve Richardson, Daniel Leventhal, Rachael M. Wolters, Cecile A. W. Geuijen, Matthew A. Sleeman, Niccolo Pengo, Francesca Rose Donnellan.
   Ann N Y Acad Sci. 2023;1519(1):153-66. DOI: 10.1111/nyas.14915

6. **Asia-Pacific International Center of Excellence in Malaria Research: Maximizing Impact on Malaria Control Policy and Public Health in Cambodia and Papua New Guinea**
   Leanne J. Robinson, Moses Laman, Leo Makita, Dysoley Lek, Annie Dori, Rachael Farquhar, Amelie Vantaux, Benoit Witkowski, Stephan Karl, Ivo Mueller.
   Am J Trop Med Hyg. 2022;107(4_Suppl):124-30. DOI: 10.4269/ajtmh.21-1324

7. **Cambodia achieved a high vaccination coverage for its population: A good example of a lower middle-income country**
   Ngoc Phuong Hong Tao, Dang Nguyen, Le Huu Nhat Minh, Veasna Duong, Chey Beaupha, Tareq Al Ahdal, Nguyen Tien Huy.
   J Glob Health. 2022;12:03088. DOI: 10.7189/jogh.12.03088

8. **Childhood encephalitis in the Greater Mekong region (the SouthEast Asia Encephalitis Project): a multicentre prospective study**
   Jean David Pommier, Chris Gorman, Yoann Crabol, Kevin Bleakley, Heng Sothy, Ky Santy, Huong Thi Thu Tran, Lam Van Nguyen, Em Bunnakea, Chaw Su Hlaing, Aye Mya Min Aye, Julien Cappelle, Magali Herrant, Patrice Piola, Bruno Rosset, Veronique Chevalier, Arnaud Tarantola, Mey Channa, Jerome Honnorat, Anne Laure Pinto, Sayaphet Rattanavong, Manivanh Vongsouvath, Mayfong Mayxay, Sommanikhone Phangmanixay, Khounthavy Phongsavath, Ommar Swe Tin, Latt Latt Kyaw, Htay Htay Tin, Kyaw Linn, Thi Mai Hung Tran, Philippe Pérot, Nguyen Thi Thu Thuy, Nguyen Hien, Phuc Huu Phan, Philippe Buchy, Philippe Dussart, Denis Laurent, Marc Eloit, Audrey Dubot-Pérès, Olivier Lortholary, Xavier de Lamballerie, Paul N Newton, Marc Lecuit, **SEAe Consortium:** Philippe Buchy, Em Bunnakea, Julien Cappelle, Mey Channa, Veronique Chevalier, Yoann Crabol, Xavier de Lamballerie, Audrey Dubot-Pérès, Philippe Dussart, Marc Eloit, Chris Gorman, Magali Herrant, Nguyen Hien, Chaw Su Hlaing, Jérôme Honnorat, Tran Thi Mai Hung, Tran Thi Thu Huong, Latt Latt Kyaw, Nguyen Van Lam, Denis Laurent, Marc Lecuit, Kyaw Linn, Olivier Lortholary, Mayfong Mayxay, Aye Mya Min Aye, Paul Newton, Philippe Perot, Sommanikhone Phangmanixay, Khounthavy Phongsavath, Phan Huu Phuc, Anne-Laurie Pinto, Patrice Piola, Jean-David Pommier, Sayaphet Rattanavong, Bruno Rosset, Ky Santy, Heng Sothy, Arnaud Tarantola, Nguyen Thi Thu Thuy, Htay Htay Tin, Ommar Swe Tin, Manivanh Vongsouvath, Pham Nhat An, Dang Duc Anh, Pascal Bonnet, Kimrong Bun, Danoy Chommanam, Viengmon Davong, Patrice Debré, Jean-François Delfraissy, Christian Devaux, Anousone Douangnouvong, Veasna Duong, Benoit Durand, Chanreaksmey Eng, Catherine Ferrant, Didier Fontenille, Lukas Hafner, Le Thanh Hai, Do Thu Huong, Marc Jouan, May July, Magali Lago, Jean-Paul Moatti, Bernadette Murgue, Khin Yi Oo, MengHeng Oum, Khansoudaphone Phakhounthong, Anh Tuan Pham, Do Quyen, Malee Seephonelee, Maud Seguy, Bountoy Sibounheunang, Kanarith Sim, Luong Minh Tan, Cho Thair, Win Thein, Phung Bich Thuy, Hervé Tissot-Dupont, Malavanh Vongsouvath.
   The Lancet Global Health. 2022;10(7):e989-1002. DOI: 10.1016/S2214-109X(22)00174-7

9. **Culex vishnui**
   Pierre-Olivier Maquart, Sébastien Boyer.
   Trends in Parasitology. 2022;38(6):491-2. DOI: 10.1016/j.pt.2022.01.003

10. ***Culex vishnui* (Diptera: Culicidae): An Overlooked Vector of Arboviruses in South-East Asia**
    Pierre-Olivier Maquart, Leakena Chann, Sebastien Boyer.
    Journal of Medical Entomology. 2022;59(4):1144-53. DOI: 10.1093/jme/tjac044

11. **Dengue virus NS1 protein conveys pro-inflammatory signals by docking onto high-density lipoproteins**
    Souheyla Benfrid, Kyu-Ho Park, Mariano Dellarole, James E Voss, Carole Tamietti, Gérard Pehau-Arnaudet, Bertrand Raynal, Sébastien Brûlé, Patrick England, Xiaokang Zhang, Anastassia Mikhailova, Milena Hasan, Marie-No€elle Ungeheuer, Stéphane Petres, Scott B Biering, Eva Harris,

Anavaj Sakuntabhai, Philippe Buchy, Veasna Duong, Philippe Dussart, Fasséli Coulibaly, François Bontems, Felix A Rey, Marie Flamand.
EMBO reports. John Wiley & Sons, Ltd; 2022;n/a(n/a):e53600. DOI: 10.15252/embr.202153600

12. **Development of a Clinical Prediction Score Including Monocyte-to-Lymphocyte Ratio to Inform Tuberculosis Treatment Among Children With HIV: A Multicountry Study**
    Amyn A. Malik, Neel R. Gandhi, Olivier Marcy, Elisabetta Walters, Mathurin Tejiokem, Giang Do Chau, Saad B. Omer, Timothy L. Lash, Mercedes C. Becerra, Irene N. Njuguna, Sylvia M. LaCourse, Elizabeth Maleche-Obimbo, Dalton Wamalwa, Grace C. John-Stewart, Lisa M. Cranmer.
    Open Forum Infect Dis. 2022;9(11):ofac548. DOI: 10.1093/ofid/ofac548

13. **Diagnostic Advances in Childhood Tuberculosis—Improving Specimen Collection and Yield of Microbiological Diagnosis for Intrathoracic Tuberculosis**
    Eric Wobudeya, Maryline Bonnet, Elisabetta Ghimenton Walters, Pamela Nabeta, Rinn Song, Wilfred Murithi, Walter Mchembere, Bunnet Dim, Jean-Voisin Taguebue, Joanna Orne-Gliemann, Mark P. Nicol, Olivier Marcy.
    Pathogens. 2022;11(4):389. DOI: 10.3390/pathogens11040389

14. **Early Changes in Interferon Gene Expression and Antibody Responses Following Influenza Vaccination in Pregnant Women.**
    Raquel Giacomelli Cao, Lisa Christian, Zhaohui Xu, Lisa Jaramillo, Bennett Smith, Erik A Karlsson, Stacey Schultz Cherry, Asuncion Mejias, Octavio Ramilo.
    The Journal of Infectious Diseases. 2022;225(2):341-51. DOI: 10.1093/infdis/jiab345

15. **Editorial: Balanced and Unbalanced Immune Response to Dengue Virus in Disease Protection and Pathogenesis**
    Tineke Cantaert, Nolwenn Jouvenet, Sean A. Diehl.
    Frontiers in Immunology [En ligne]. 2022 [cité le 9 mai 2022];13. Disponible: https://www.frontiersin.org/article/10.3389/fimmu.2022.835731

16. **Effect of systematic tuberculosis detection on mortality in young children with severe pneumonia in countries with high incidence of tuberculosis: a stepped-wedge cluster-randomised trial**
    Olivier Marcy, Eric Wobudeya, Hélène Font, Aurélia Vessière, Chishala Chabala, Celso Khosa, Jean-Voisin Taguebue, Raoul Moh, Juliet Mwanga-Amumpaire, Manon Lounnas, Veronica Mulenga, Sandra Mavale, Josina Chilundo, Dalila Rego, Bwendo Nduna, Perfect Shankalala, Uzima Chirwa, Agathe De Lauzanne, Bunnet Dim, Emeline Tiogouo Ngouana, Madeleine Folquet Amorrissani, Lassina Cisse, Flore Amon Tanoh Dick, Eric A. Komena, Sylvie Kwedi Nolna, Gerald Businge, Naome Natukunda, Saniata Cumbe, Prossy Mbekeka, Ang Kim, Chanrithea Kheang, Sokha Pol, Elizabeth Maleche-Obimbo, James A. Seddon, Tan Eang Mao, Stephen M. Graham, Christophe Delacourt, Laurence Borand, Maryline Bonnet, Olivier Marcy, Angeline Serre, Anne Badrichani, Manoa Razafimanantsoa, Julien Poublan, Aurélia Vessière, Clémentine Roucher, Estelle Occelli, Aurélie Beuscart, Aurélie Charpin, Gemma Habiyambere, Salomé Mesnier, Eric Balestre, Bandana Bhatta, Anne-Laure Maillard, Joanna Orne-Gliemann, Emmanuelle Baillet, Nicolas Koskas, Marc D'Elbée, Delphine Gabillard, Hélène Font, Minh Huyen, Maryline Bonnet, Manon Lounnas, Hélène Espérou, Sandrine Couffin-Cadiergues, Alexis Kuppers, Benjamin Hamze, Laurence Borand, Agathe de Lauzanne, Bunnet Dim, Chanthy Keang, Long Pring, Song Yin, Channimol

Sarith, Chanvirak Phan, Sovann Nheuong, Socheat Ly, Sanary Kaing, Vouchleang Sreng, Elen Lun, Leakhena Say, Sophea Suom, Romyka Ferhy, Dina So, Sorunna Born, Sophea Pal, Boraneath Nang, Tan Eang Mao, Ang Kim, Viso Srey, Piseth Kan, Leakhena Hout, Samnang Ith, Sophany Oum, Sokunvadhana Sau, Kim Heang Ho, Daronic Kith, Nathara Nuch, Chhun Leang Horm, Cheameas Sophon, Bosba Roeungdeth, Chhay Meng, Ravin Rith, Samnang Phy, Chanchetra Sor, Voleak Sao, Sophea Khat, Bunthoeun Mak, Angkeaborin Uy, Sreyny Khay, Kimsan Som, Rongvirak Hach, Hay Sok, Sotheavy Kuon, Synatt Heng, Amara Seng, Sopheak Nim, Reach Pan, Srean Kim, Keo SREY Leap, Bormey Net, Viccheka Noun, Daven Lay, Chhaing Many, Socheata Seng, Vuthy Ly, Saran So, Sovutthik Oun, Sopheap Chey, Rattany Chhea, Lydeth Baong, Vanna Thoung, Chanrithea Kheang, Borady By, Vathanak Nguon, Eksophea Meach, Sopheak Tek, Sina Ngeav, Tetra Lun, Deth Hem, Nayreang Chut, Setha Sarik, Hgekkoung Nang, Mengnean Meach, Sopal Sreng, Dara Sar, Rathana Kin, Phoran Ros, Chenda Dorn, Chansy Kak, Srey Leak Sambath, Leakhena Son, Linda Bin, Eangnay Pengong, Sokha Pol, Samnang Khutsorn, Sorsophea Seang, Virak Soun, Vuthy Vong, Chandara Khoeung, Panha Um, Sokunthea Bou, Sarin Song Pich, Puthy Nim, Sopheak Khat, Nuon Ban Si, Sovannodom Ream, Sim Ing, Phanith Chann, Samrith Ngeth, Marina Sun, Sokea Chhoeung, Soeun Sean, Ratanak Prak, Jean-Voisin Taguebue, Sylvie Kwedi Nolna, Audrey Amboua Schouame Onambele, Numfor Hycenth, Bernard Melingui, Angeline Nkembe Medounmga, Luciole Hougnang Tatmi, Nathalie Etemgoua, Vanessa Kouesso, Jean Bugin, Celestine Nzedjom, Roger Ngoya, Jules Eyike, Elyse Loudjom, Roger Lonsti, Ladi Dang, Edward Bintar, Chantal Njayong, Cinthia Ngonsoa O, Isabelle Ndzeukap, Pascaline Dzoyem, Clémentine Dzokou, Berthe Dindo, Raoul Moh, Eric Auguste Komena, Roger Aka Bony, Christian Kouadio, Serge Danho, Melissa Goli, Madeleine Folquet, Max Valère Itchy, Abdel Sidibé, Lancina Cissé, Joseph Ouattara, Mamadou Konaté, Flore Amon-Tanoh Dick, Melissa Cardena, Laurence Adonis-Koffi, Djabia Eugenie, Ferdinand Kouamé, Hervé Menan, André Inwoley, Timothée Ouassa, Marcelle Sandrine Nguessan, Celso Khosa, Saniata Cumbe, Emelva Manhiça, Alcina Zitha, Valter Chiúle, Eva Muxanga, Irene Gune, Yara Lima, Jorge Ribeiro, Sandra Mavale, Josina Chilundo, Felismina Maxanguana, Natália Morais, Julieta Manhiça, Josefina Give, Jafito Atumane, Gelson Lucas, Arsénio Thai, Adélio Chave, Dalila Rego, Lúcia Guambe, Faiaz Issa, Rosa Carneiro, Neusa Pene, Natércia Florindo, Dália Machel, Cecília Cumbane, Helena Mendes, Mule Kitungwa, Valdo Muianga, Humberto Tamele, Adelino Sulude, Roda Mabota, Herquéria Comandante, Abelardo Massangaie, Eric Wobudeya, Gerald Bright Businge, Faith Namulinda, Robert Sserunjogi, Rashidah Nassozi, Charlotte Barungi, Hellen Aanyu, Doreen Muwonge, Eva Kagoya, Serene Aciparu, Sophia Chemutai, Samuel Ntambi, Amir Wasswa, Juliet Nangozi, Abner Tagoola, Prossy Mbekeka, Sajja Kenneth, John Paul Lubega, Aidah Nassali, Jessica Tagobera, Christine Agwang, Florence Kalembe, Annet Ajambo, Elizabeth Aguti, Samuel Kasibante, Henry Matende, Israel Owen Odongo, Juliet Mwanga Amumpaire, Naome Natukunda, Gertrude Ngabirano, Paul Kakwenza, Simpson Nuwamanya, Miria Nyangoma, Jane Nabbuto, Florence Abok, Rinah Arinaitwe, Diana Birungi, Evans Mwesigwa, Daniel Atwine, Hassan Mbega, Patrick Orikiriza, Ivan Taremwa, Esther Turyashemererwa, Hope Derrick, Dan Nyehangane, Rodney Kaitano, Susan Logoose, Steven Businge, Charles Ntambi, Jerome Mugabi, John Mzee, Julius Besigye, Saul Kanzira, Phionah Turyatemba, Florence Twebaze, Chishala Chabala, Veronica Mulenga, Perfect Shankalala, Chimuka Hambulo, Vincent Kapotwe, Marjory Ngambi, Kunda Kasakwa, Uzima Chirwa, Chifunda Kapula, Susan Zulu, Grace Nawakwi, Teddy Siasulingana, Jessy Chilonga, Maria Chimbini, Mutinta Chilanga, Bwendo Nduna, Muleya Inambao, Mwate Mwambazi, Barbra Halende, Wyclef Mumba, Endreen Mankunshe, Maureen Silavwe, Moses Chakopo, Roy Moono.
The Lancet Infectious Diseases. Elsevier; 2022;0(0). DOI: 10.1016/S1473-3099(22)00668-5

17. **Efficacy of three anti-malarial regimens for uncomplicated Plasmodium falciparum malaria in Cambodia, 2009-2011: a randomized controlled trial and brief review**
    Dysoley Lek, Agus Rachmat, Dustin Harrison, Geoffrey Chin, Suwanna Chaoratanakawee, David Saunders, Didier Menard, William O. Rogers.
    Malar J. 2022;21(1):259. DOI: 10.1186/s12936-022-04279-3

18. **Evaluation of cutaneous immune response in a controlled human in vivo model of mosquito bites**
    David Guerrero, Hoa Thi My Vo, Chanthap Lon, Jennifer A. Bohl, Sreynik Nhik, Sophana Chea, Somnang Man, Sokunthea Sreng, Andrea R. Pacheco, Sokna Ly, Rathanak Sath, Sokchea Lay, Dorothée Missé, Rekol Huy, Rithea Leang, Hok Kry, Jesus G. Valenzuela, Fabiano Oliveira, Tineke Cantaert**, Jessica E. Manning**.
    Nat Commun. Nature Publishing Group; 2022;13(1):7036. DOI: 10.1038/s41467-022-34534-9

19. **Evaluation of HRP2 and pLDH-based rapid diagnostic tests for malaria and prevalence of pfhrp2/3 deletions in Aweil, South Sudan**
    Emily Lynch, Tomas O. Jensen, Bachir Assao, Menard Chihana, Thadeous Turuho, Dan Nyehangane, John B. Manyok, Harriet Pasquale, Nimol Khim, Benoit Witkowski, Matthew E. Coldiron.
    Malar J. 2022;21(1):261. DOI: 10.1186/s12936-022-04280-w

20. **Evaluation of Zika rapid tests as aids for clinical diagnosis and epidemic preparedness**
    Debi Boeras, Cheikh Tidiane Diagne, Jose L. Pelegrino, Marc Grandadam, Veasna Duong, Philippe Dussart, Paul Brey, Didye Ruiz, Marisa Adati, Annelies Wilder-Smith, Andrew K. Falconar, Claudia M. Romero, Maria Guzman, Nagwa Hasanin, Amadou Sall, Rosanna W. Peeling.
    eClinicalMedicine. 2022;49:101478. DOI: 10.1016/j.eclinm.2022.101478

21. **Excess risk of subsequent infection in hospitalized children from a community cohort study in Cambodia and Madagascar**
    Lison Rambliere, Elsa Kermorvant-Duchemin, Agathe de Lauzanne, Jean-Marc Collard, Perlinot Herindrainy, Muriel Vray, Benoit Garin, Andrianirina Zafitsara Zo, Fanjalalaina Rasoanaivo, Jacob Rakotoarimanana Feno Manitra, Tanjona Bodonirina Raheliarivao, Jean-Baptiste Niokhhor Diouf, Véronique Ngo, Siyin Lach, Pring Long, Laurence Borand, Touch Sok, Armiya Youssouf Abdou, Michael Padget, Yoann Madec, Didier Guillemot, Elisabeth Delarocque-Astagneau, Bich-Tram Huynh.
    International Journal of Epidemiology. 2022;dyac048. DOI: 10.1093/ije/dyac048

22. **Genomic Shifts, Phenotypic Clines, and Fitness Costs Associated With Cold Tolerance in the Asian Tiger Mosquito**
    Stéphanie Sherpa, Jordan Tutagata, Thierry Gaude, Frédéric Laporte, Shinji Kasai, Intan H. Ishak, Xiang Guo, Jiyeong Shin, Sébastien Boyer, Sébastien Marcombe, Theeraphap Chareonviriyaphap, Jean-Philippe David, Xiao-Guang Chen, Xiaohong Zhou, Laurence Després.
    Molecular Biology and Evolution. 2022;39(5):msac104. DOI: 10.1093/molbev/msac104

23. **Household clustering supports a novel chemoprophylaxis trial design for a mosquito-borne viral disease**
    Hugh R. Watson, Veasna Duong, Sowath Ly, Marie Mandron, André M. Siqueira, Guilherme S. Ribeiro.
    Int J Infect Dis. 2022;122:169-73. DOI: 10.1016/j.ijid.2022.05.020

24. **Immunoglobulin-free strategy to prevent HBV mother-to-child transmission in Cambodia (TA-PROHM): a single-arm, multicentre, phase 4 trial**
    Olivier Segeral, Bunnet Dim, Christine Durier, Sovann Nhoueng, Kearena Chhim, Saren Sovann, Sophal Yom, Chanlina Vong, Song Yin, Bandith Ros, Vutha Ky, Sothy Pech, Bunthoeun Nem, Kay Hout, Julia Guillebaud, Eamkim Ear, Layana Caroupaye-Caroupin, Claire Rekacewicz, Laura Fernandez, Denis Laurent, Chantana Yay, Rattana Kim, Laurence Meyer, Samsorphea Chhun, **Laurence Borand for the ANRS-MIE TA PROHM Study Group** Chanthy Keang, Ousa Khan, Boraneath Nang, Vouch Leang Sreng, Sopheavet In, Sineath Sun, Linda Sov, Bunrachana Nor, Brembrey Hing, Sokkim Seng, Sophea Soum, Leakhena Say, Sao Sarady Ay, Daneth Thol, Chhorn Chhouk, Patrice Piola, Janin Nouhin, Anne-Marie Roque Afonso, Jean Charles Duclos Vallee, Channa Sann, Leang Sim Kruy, Maud Lemoine, Laurent Mandelbrot, Stephane Blanche, Alpha Diallo, Christelle Paul, SAY Tiv, Polinn Sar, Lyvoin Nov, Darapoline Vann, Tha Chea, Bunrith Touch, Kongkea Neav, Ekvitou Kong, Ratha Chea, Chanksolina Ouk, Lyhour Meak, Rayounette Krouch, Naneth Chhan, Sody Seang, Veasna Nuon, Leang Meng, Sok Leakhena Tharith, Sovannara Hang, Vanrithy Som, Rithy Som, Phirak Seng, Malys Lim, Kimchhorn Srey, Sok Rothavy Uch, Pichthyda Hou, Satha Bo, Eanghor Ieang, Kimchhorng Korn, Chan Reatrey Noun, Sokhoeun Soy, Thou Khim, Vutha Sou, Sokha Pol, Samreth Nget, Marina Sun, Phearom Uon, Kim Teng Ya, Kimsreng Lean, Kim Ean Eang, Sophal Ung, Rauin Rith, Charya Mom, Chanthea Keang, Soklyda Sam, Sokneth Chuong, Chanmony Nam, Sophya Khuon, Sidet Cheang, Sopheak Lean, Arnaud Tarantola, Isabelle Fournier, Nicolas Rouveau, Maria-Camila Calvo cortez.
    The Lancet Infectious Diseases. 2022; DOI: 10.1016/S1473-3099(22)00206-7

25. **Impact of the switch from four to three intradermal rabies post-exposure prophylaxis sessions in patients bitten by dogs: A cost-consequence analysis from the patients' perspective**
    Alicia Le Bras, Kevin Zarca, Yiksing Peng, Malen Chan, Isabelle Durand-Zaleski.
    One Health. 2022;15:100408. DOI: 10.1016/j.onehlt.2022.100408
26. **Influx of Backyard Farming with Limited Biosecurity Due to the COVID-19 Pandemic Carries an Increased Risk of Zoonotic Spillover in Cambodia**
    Sudipta Hyder, Benjamin L. Sievers, Claude Flamand, Damian TagoPacheco, Malen Chan, Filip Claes, Erik A. Karlsson.
    Microbiol Spectr. 2022;e0420722. DOI: 10.1128/spectrum.04207-22

27. **Knowledge, attitudes and practices on childhood TB among healthcare workers**
    B. Joshi, H. Font, E. Wobudeya, M. Nanfuka, A. Kobusingye, J. Mwanga-Amumpaire, N. Natukunda, S. Turyahabwe, L. Borand, T. E. Mao, B. Dim, R. Ferhi, R. Moh, J. Kouakou, R. Aka Bony, G. Breton, A. Mustapha, L. Matata, L. Foray, A. Detjen, S. Verkuijl, M. Sekadde, C. Khosa, V. Mbassa, J-V. Taguebue, S. Kwedi Nolna, M. Bonnet, O. Marcy, J. Orne-Gliemann.
    Int j tuberc lung dis. 2022;26(3):243-51. DOI: 10.5588/ijtld.21.0317

28. **MALDI-TOF MS: An effective tool for a global surveillance of dengue vector species**
    Antsa Rakotonirina, Morgane Pol, Fara Nantenaina Raharimalala, Valentine Ballan, Malia Kainiu, Sébastien Boyer, Sosiasi Kilama, Sébastien Marcombe, Sylvie Russet, Emilie Barsac, Rama

Vineshwaran, Malia Kaleméli Selemago, Vincent Jessop, Geneviève Robic, Romain Girod, Paul T. Brey, Julien Colot, Myrielle Dupont-Rouzeyrol, Vincent Richard, Nicolas Pocquet.
PLoS One. 2022;17(10):e0276488. DOI: 10.1371/journal.pone.0276488

29. Metabolic, Pharmacokinetic, and Activity Profile of the Liver Stage Antimalarial (RC-12)

Yuxiang Dong, Yogesh Sonawane, Steven P. Maher, Anne-Marie Zeeman, Victor Chaumeau, Amélie Vantaux, Caitlin A. Cooper, Francis C. K. Chiu, Eileen Ryan, Jenna McLaren, Gong Chen, Sergio Wittlin, Benoît Witkowski, François Nosten, Kamaraj Sriraghavan, Dennis E. Kyle, Clemens H. M. Kocken, Susan A. Charman, Jonathan L. Vennerstrom.
ACS Omega. 2022;7(14):12401-11. DOI: 10.1021/acsomega.2c01099

30. Modelling Japanese encephalitis virus transmission dynamics and human exposure in a Cambodian rural multi-host system

Héléna Ladreyt, Véronique Chevalier, Benoit Durand.
PLoS Negl Trop Dis. 2022;16(7):e0010572. DOI: 10.1371/journal.pntd.0010572

31. Monitoring insecticide resistance of adult and larval Aedes aegypti (Diptera: Culicidae) in Phnom Penh, Cambodia

Sebastien Boyer, Pierre-Olivier Maquart, Kalyan Chhuoy, Kimhuor Suor, Moeun Chhum, Kimly Heng, Sokkeang Leng, Didier Fontenille, Sebastien Marcombe
Parasites & Vectors. 2022;15(1):44. DOI: 10.1186/s13071-022-05156-3

32. Mosquito (Diptera: Culicidae) diversity and medical importance in Koh Kong mangrove forests, Cambodia

Pierre-Olivier Maquart, Chea Sokha, Sébastien Boyer.
Asian Biomedicine. 2022;16(3):121-9. DOI: 10.2478/abm-2022-0015

33. Non-toxigenic Corynebacterium diphtheriae in hallux ulceration

Gauthier Delvallez, Edgar Badell, Sokleaph Cheng, Soda Meng, Vivandet Tong, Judy Norman, Julie Toubiana, Koen Vandelannoote, Anne-Laure Bañuls, Mallorie Hide, Sylvain Brisse.
J Infect Dev Ctries. 2022;16(06):1118-21. DOI: 10.3855/jidc.16153

34. Outdoor residual spraying for malaria vector-control in Kayin (Karen) state, Myanmar: A cluster randomized controlled trial

Victor Chaumeau, Ladda Kajeechiwa, Thithiworada Kulabkeeree, Sunisa Sawasdichai, Warat Haohankhunnatham, Aritsara Inta, Monthicha Phanaphadungtham, Florian Girond, Vincent Herbreteau, Gilles Delmas, François Nosten.
PLoS One. 2022;17(9):e0274320. DOI: 10.1371/journal.pone.0274320

35. Pestilence and famine: Continuing down the vicious cycle with COVID-19

Sudipta Hyder, Rethy K. Chhem, Filip Claes, Erik Albert Karlsson.
PLOS Pathogens. Public Library of Science; 2022;18(10):e1010810. DOI: 10.1371/journal.ppat.1010810

36. Plastic pollution and infectious diseases

Pierre-Olivier Maquart, Yves Froehlich, Sebastien Boyer.
The Lancet Planetary Health. Elsevier; 2022;6(10):e842-5. DOI: 10.1016/S2542-5196(22)00198-X

37. **Preclinical characterization and target validation of the antimalarial pantothenamide MMV693183**

    Laura E. de Vries, Patrick A. M. Jansen, Catalina Barcelo, Justin Munro, Julie M. J. Verhoef, Charisse Flerida A. Pasaje, Kelly Rubiano, Josefine Striepen, Nada Abla, Luuk Berning, Judith M. Bolscher, Claudia Demarta-Gatsi, Rob W. M. Henderson, Tonnie Huijs, Karin M. J. Koolen, Patrick K. Tumwebaze, Tomas Yeo, Anna C. C. Aguiar, Iñigo Angulo-Barturen, Alisje Churchyard, Jake Baum, Benigno Crespo Fernández, Aline Fuchs, Francisco-Javier Gamo, Rafael V. C. Guido, María Belén Jiménez-Diaz, Dhelio B. Pereira, Rosemary Rochford, Camille Roesch, Laura M. Sanz, Graham Trevitt, Benoit Witkowski, Sergio Wittlin, Roland A. Cooper, Philip J. Rosenthal, Robert W. Sauerwein, Joost Schalkwijk, Pedro H. H. Hermkens, Roger V. Bonnert, Brice Campo, David A. Fidock, Manuel Llinás, Jacquin C. Niles, Taco W. A. Kooij, Koen J. Dechering.

    Nat Commun. 2022;13(1):2158. DOI: 10.1038/s41467-022-29688-5

38. **Predicting the Effects of Climate Change on Dengue Vector Densities in Southeast Asia through Process-Based Modeling**

    Lucas Bonnin, Annelise Tran, Vincent Herbreteau, Sébastien Marcombe, Sébastien Boyer, Morgan Mangeas, Christophe Menkes. Environmental Health Perspectives. Environmental Health Perspectives 130(12):127002. DOI: 10.1289/EHP11068

39. **Predicting the potential for zoonotic transmission and host associations for novel viruses**

    P. S. Pandit, S. J. Anthony, T. Goldstein, K. J. Olival, M. M. Doyle, N. R. Gardner, B. Bird, W. A. Smith, D. Wolking, K. Gilardi, C. Monagin, T. Kelly, M. Uhart, J. H. Epstein, C. Machalaba, M. K. Rostal, P. Dawson, E. Hagan, A. Sullivan, H. Li, A. A. Chmura, A. Latinne, C. Lange, T. O'Rourke, S. H. Olson, L. Keatts, A. P. Mendoza, A. Perez, C. Dejuste de Paula, D. Zimmerman, M. Valitutto, M. LeBreton, D. McIver, A. Islam, V. Duong, M. Mouiche, Z. Shi, P. Mulembakani, C. Kumakamba, M. Ali, N. Kebede, U. Tamoufe, S. Bel-Nono, A. Camara, J. Pamungkas, K. Coulibaly, E. Abu-Basha, J. Kamau, S. Silithammavong, J. Desmond, T. Hughes, E. Shiilegdamba, O. Aung, D. Karmacharya, J. Nziza, D. Ndiaye, A. Gbakima, Z. Sijali, S. Wacharapluesadee, E. Alandia Robles, B. Ssebide, G. Suzán, L. F. Aguirre, M. R. Solorio, T. N. Dhole, N. T. T. Nga, P. L. Hitchens, D. O. Joly, K. Saylors, A. Fine, S. Murray, W. Karesh, P. Daszak, J. A. K. Mazet, PREDICT Consortium, C. Dejuste de Paula, C. K. Johnson.

    Commun Biol. 2022;5(1):844. DOI: 10.1038/s42003-022-03797-9

40. **Presence of Recombinant Bat Coronavirus GCCDC1 in Cambodian Bats**

    Feng Zhu*, Veasna Duong*, Xiao Fang Lim, Vibol Hul, Tanu Chawla, Lucy Keatts, Tracey Goldstein, Alexandre Hassanin, Vuong Tan Tu, Philippe Buchy, October M. Sessions, Lin-Fa Wang, Philippe Dussart, Danielle E. Anderson.

    Viruses. 2022;14(2):176. DOI: 10.3390/v14020176

41. **Prevalence and factors associated with faecal carriage of extended-spectrum β-lactamase-producing Enterobacterales among peripartum women in the community in Cambodia**

    Agathe de Lauzanne, Navin Sreng, Elsa Foucaud, Touch Sok, Thida Chon, Chhaily Yem, Veasna Hak, Sothada Heng, Meng Soda, Malika Gouali, Maya Nadimpalli, Malin Inghammar, Mamitina Alain Noah Rabenandrasana, Jean Marc Collard, Muriel Vray, Simon Le Hello, Alexandra Kerleguer, Patrice Piola, Elisabeth Delarocque-Astagneau, Didier Guillemot, Bich Tram Huynh, Laurence Borand, **BIRDY study group**: Andrianirina Zafitsara Zo, Marguerite Diatta, Jean Baptiste Diouf,

Fatoumata Diene Sarr, Joseph Faye, Sophie Goyet, Perlinot Herindrainy, Elsa Kermorvant-Duchemin, Siyin Lach, Veronique Ngo, Michael Padget, Feno Manitra Jacob Rakotoarimanana, Bodonirina Tanjona Raheliarivao, Frédérique Randrianirina, Abdoulaye Seck, Arnaud Tarantola, Armya Abdou Youssouf.
Journal of Antimicrobial Chemotherapy. 2022;dkac224. DOI: 10.1093/jac/dkac224

42. Rapid Generation of In-House Serological Assays Is Comparable to Commercial Kits Critical for Early Response to Pandemics: A Case With SARS-CoV-2
Heidi Auerswald, Chanreaksmey Eng, Sokchea Lay, Saraden In, Sokchea Eng, Hoa Thi My Vo, Charya Sith, Sokleaph Cheng, Gauthier Delvallez, Vann Mich, Ngy Meng, Ly Sovann, Kraing Sidonn, Jessica Vanhomwegen, Tineke Cantaert, Philippe Dussart, Veasna Duong, Erik A. Karlsson.
Front Med. 2022;9:864972. DOI: 10.3389/fmed.2022.864972

43. Robust and Functional Immune Memory Up to 9 Months After SARS-CoV-2 Infection: A Southeast Asian Longitudinal Cohort
Hoa Thi My Vo*, Alvino Maestri*, Heidi Auerswald, Sopheak Sorn, Sokchea Lay, Heng Seng, Sotheary Sann, Nisa Ya, Polidy Pean, Philippe Dussart, Olivier Schwartz, Sovann Ly, Timothée Bruel, Sowath Ly, Veasna Duong, Erik A. Karlsson**, Tineke Cantaert**.
Frontiers in Immunology [En ligne]. 2022 [cité le 1 mars 2022];13. Disponible: https://www.frontiersin.org/article/10.3389/fimmu.2022.817905

44. Seroprevalence of brucellosis, Q fever and Rift Valley fever in domestic ruminants in Guinea in 2017–2019
Cécile Troupin, Isabelle Ellis, Bakary Doukouré, Alimou Camara, Moustapha Keita, Moise Kagbadouno, Jean-Mathieu Bart, Ramadan Diallo, Sandra Lacôte, Philippe Marianneau, Martin H Groschup, Noël Tordo.
BMC Vet Res. 2022;18(1):64. DOI: 10.1186/s12917-022-03159-x

45. Single-cell RNA sequencing of Plasmodium vivax sporozoites reveals stage- and species-specific transcriptomic signatures
Anthony A. Ruberto, Caitlin Bourke, Amélie Vantaux, Steven P. Maher, Aaron Jex, Benoit Witkowski, Georges Snounou, Ivo Mueller.
PLoS Negl Trop Dis. 2022;16(8):e0010633. DOI: 10.1371/journal.pntd.0010633

46. Small-scale field assessment against the dengue vector Aedes aegypti using the auto-dissemination approach in an urban area of Vientiane, Lao PDR
Phoutmany Thammavong, Sebastien Boyer, Phonesavanh Luangamath, Nothasine Phommavanh, Vaekey Vungkyly, Somphat Nilaxay, Khaithong Lakeomany, Paul Brey, Marc Grandadam, Sebastien Marcombe.
PLoS ONE. 2022;17(7):e0270987. DOI: 10.1371/journal.pone.0270987

47. Spirulina as a daily nutritional supplement of young pre-school Cambodian children of deprived settings: a single-blinded, placebo-controlled, cross-over trial
Hubert Barennes, Laetitia Houdart, Caroline de Courville, Florent Barennes.
BMC Pediatr. 2022;22(1):701. DOI: 10.1186/s12887-022-03766-5

48. **Temporal patterns of functional anti-dengue antibodies in dengue infected individuals with different disease outcome or infection history.**
    Hoa Thi My Vo, Vinit Upasani, Heidi Auerswald, Sokchea Lay, Sotheary Sann, Axelle Vanderlinden, Sreymom Ken, Sopheak Sorn, Sowath Ly, Veasna Duong, Philippe Dussart, Tineke Cantaert.
    Sci Rep. Nature Publishing Group; 2022;12(1):17863. DOI:10.1038/s41598-022-21722-2

49. **TLR2 axis on peripheral blood mononuclear cells regulates inflammatory responses to non-infectious immature dengue virus particles.**
    José Alberto Aguilar Briseño, Lennon Ramos Pereira, Marleen van der Laan, Mindaugas Pauzuolis, Bram M. Ter Ellen, Vinit Upasani, Jill Moser, Luís Carlos de Souza Ferreira, Jolanda M. Smit, Izabela A. Rodenhuis-Zybert.
    PLoS Pathog. 2022;18(10):e1010499. DOI: 10.1371/journal.ppat.1010499

50. **Tuberculosis Diagnosis in HIV-Infected Children: Comparison of the 2012 and 2015 Clinical Case Definitions for Classification of Intrathoracic Tuberculosis Disease.**
    Olivier Marcy, Sophie Goyet, Laurence Borand, Philippe Msellati, Vibol Ung, Mathurin Tejiokem, Giang Do Chau, Francis Ateba-Ndongo, Abdoul Salam Ouedraogo, Bunnet Dim, Paul Perez, Julien Asselineau, Guislaine Carcelain, Stéphane Blanche, Christophe Delacourt, Sylvain Godreuil.
    Journal of the Pediatric Infectious Diseases Society. 2022;11(3):108-14. DOI: 10.1093/jpids/piab113

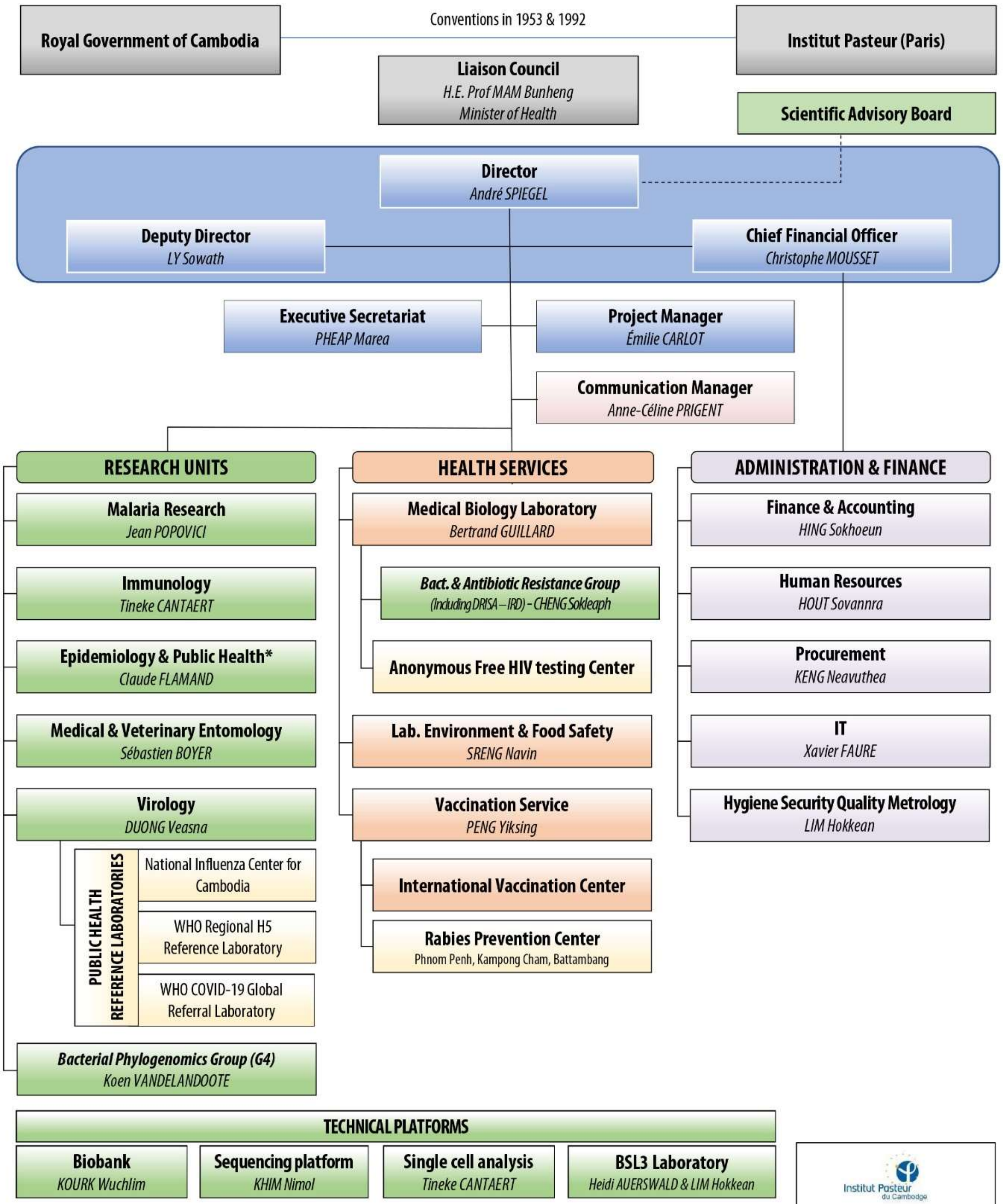
51. **Variations in the carapace shape of whip spiders (Arachnida: Amblypygi)**
    Florian Réveillion, Sophie Montuire, Pierre-Olivier Maquart, Charlotte Fétiveau, Loïc Bollache.
    Journal of Morphology. 2022;283(8):1003-14. DOI: 10.1002/jmor.21485

52. **Xenopsylla cheopis (rat flea)**
    Sebastien Boyer, Thomas R. Gillespie, Adélaïde Miarinjara.
    Trends in Parasitology. 2022;38(7):607-8. DOI: 10.1016/j.pt.2022.03.006

53. **Zika vector competence data reveals risks of outbreaks: the contribution of the European ZIKAlliance project**
    Thomas Obadia, Gladys Gutierrez-Bugallo, Veasna Duong, Ana I. Nuñez, Rosilainy S. Fernandes, Basile Kamgang, Liza Hery, Yann Gomard, Sandra R. Abbo, Davy Jiolle, Uros Glavinic, Myrielle Dupont-Rouzeyrol, Célestine M. Atyame, Nicolas Pocquet, Sébastien Boyer, Catherine Dauga, Marie Vazeille, André Yébakima, Michael T. White, Constantianus J. M. Koenraadt, Patrick Mavingui, Anubis Vega-Rua, Eva Veronesi, Gorben P. Pijlman, Christophe Paupy, Núria Busquets, Ricardo Lourenço-de-Oliveira, Xavier De Lamballerie, Anna-Bella Failloux. Nat Commun.
    Nature Publishing Group; 2022;13(1):4490. DOI: 10.1038/s41467-022-32234-y

6. Annex

ORGANIZATIONAL CHART OF THE INSTITUT PASTEUR DU CAMBODGE



Institut Pasteur du Cambodge

Reference: Organizational Chart
Date: 5 April 2023

*Including **Community Epidemiology Group, Clinical Research Group, Methodological and Statistical Analysis Group, Geo Health Group** RD/IPC), **One Health Group** (CIRAD/IPC)