Institut Pasteur du Cambodge

PASTEUR NETWORK

ANNUAL REPORT

2024

PASTEUR NETWORK

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The Institut Pasteur du Cambodge (IPC), created in December 1953, is a non-profit research institution operating under the high patronage of the Cambodian Ministry of Health (MoH). Our mission is to contribute to the prevention and treatment of infectious diseases through research, public health activities, and training. This report presents the Institute's activities in 2024.

As of 31 December 2024, the IPC's on-site staff represented 17 different nationalities. Scientific activities are carried out by more than 35 scientists, each holding at least a PhD or a doctorate degree in medicine, veterinary medicine or pharmacy and another PhD or master's degree.

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

Research activities done in 2024 were featured in 60 articles published by scientists affiliated to IPC, appearing in peer-reviewed international journals. Among them are 30 (50%) as first or last author and 35 (58%) with an IF greater than or equal to 4.
The year 2024 saw the realization of important work carried out sometimes for several months or years by different units which led to the obtaining of various large-scale projects: PREZODE-AFRICAM, ECOMORE, RACSMEI, …

Post-exposure rabies management activities decreased slightly in 2024, by -3.8% compared to 2023, with the management of 58,203 (vs 60,476 in 2023) patients in our 3 rabies prevention centres. The risks related to rabies indeed remain high in Cambodia: 65 % of the 181 animals tested for rabies virus at the Virology Unit were positive.

The IPC plays a major role in the training of university students. Its scientists participate in curricula offered by local universities, including the University of Health Sciences (UHS) in Phnom Penh. Additionally, the IPC itself welcomes many students for internships and practical experiences. During 2024, 154 students interned at the IPC. This is higher than the 131 welcomed in 2023. Among the 154 students, 128 were Cambodian nationals, while the others were from France, Belgium, Laos, Kenya, Cameroon.

An ambitious capacity-strengthening policy for young Cambodians was put in place in 2022 (young talents at the IPC), allowing them to carry out their doctorate studies at the IPC (scholarships for non-IPC students and continued salaries for IPC personnel). This policy continued throughout 2024. As a

result, 2 Cambodian defend their PhD (Immunology and Epidemiology and Public Health Unit) and IPC hosted 15 PhD student on 31st December 2024 (9 Cambodian and 6 foreigners)

Our Health Service activities in 2024 compared well to those of 2023: (i) our Medical Biology Laboratory's activities decreased by 9.4 % (5.6 vs 6.1 million of "B"), (ii) a 4.4 % increase for the total number of tests performed by for the Laboratory of Environment and Food Safety (34,758 vs 34,246); and (iii) a 19 % increase for the total number of injections (including vaccinations and immunoglobulins) provided by the International Vaccination Centre (53,017 vs 44,374).

2024 Highlights

Human Resources

- Dr DUONG Veasna, Research Director, Head of the Virology Unit since September 2020, left the IPC at the end of the year. His resignation was the result of a personal project abroad and was not linked to any specific problems encountered at IPC. He has been appointed "Adjunct Researcher" at IPC.
- Dr. Heidi AUERSWALD, virologist from the Virology Unit, "General Scientific Manager" of BSL3 since March 2023 left IPC in September 2024.
- Ms. Najet HADRI took up her duties as Head of the Grant Office on 1st January 2024.
- Mr. Alexandre BLAISE succeeded Ms. Anne-Céline PRIGENT as Head of CPI Communications in June 2024.

Important visits at IPC

Visit of H.E. Prof. CHHEANG Ra, Minister of Health, Kingdom of Cambodia (January 26th)

On Friday January 26, 2024, the IPC was honored to receive a visit from H.E. Prof. CHHEANG Ra, Minister of Health of the Kingdom of Cambodia, accompanied by his delegation. The general situation, activities, laboratories/technical platforms and progress of the Institute were presented. It was also an opportunity to highlight the close links between the Ministry of Health and the IPC. H.E. Prof. CHHEANG Ra and his Delegation then visited various IPC facilities, including (i) the Rabies Prevention Center, (ii) the Medical Biology Laboratory, (iii) the Virology Unit and (iv) the Malaria Research Unit.

Visit of Professor Françoise BARRE-SINOUSSI, 7- 8th October

On the 7th and 8th of October 2024, we were honored to welcome Professor Françoise BARRE-SINOUSSI, Nobel Prize laureate in Physiology or Medicine in 2008, to the IPC. In a symbolic moment, during this visit, Professor BARRE-SINOUSSI stood in the same room where she received the call announcing her Nobel Prize 16 years ago, on October 7, 2008.

Visit of H.E. Igor DRIESMANS, Ambassador of the European Union, October 30th.

The IPC hosted H.E. Igor Driesmans, Ambassador of the European Union (EU), as part of a European Union-funded project in the field of biosecurity. The EU delegation was presented with the equipment funded by the EU to strengthen biosecurity: the infectious waste pre-shredder and steam sterilization system (STERIPLUSTM80) and the steam sterilizer (Matachana S1000) for the BSL-3 laboratory, along with their operating mode and advantages.

Visit of Dr Jaana Marianna TRIAS, WHO Representative to Cambodia, September 26th.

On Thursday, 26th September, the IPC welcomed Dr. Jaana Marianna Trias, WHO Representative to Cambodia accompanied by Dr. Pascal Ringwald, Coordinator of the Mekong Malaria Elimination Programme. The visit was an opportunity to discuss the very close collaboration between WHO and the IPC, particularly in terms of a joint mission to support the Cambodian MoH..

Research: research activities, support for research

AfriCam Preventing Zoonotic Diseases Emergence (PREZODE)

Through the PREZODE Initiative, Cambodia is the only Asian country involved in the AfriCam project, alongside four African countries: Cameroon, Guinea, Madagascar, and Senegal. This project, running to 2026, aimed to study how hydrological dynamics, climate, and environmental factors influence the risk of zoonotic disease emergence across diverse ecosystems. These ecosystems represent key interfaces between humans, animals, and the environment.

The AfriCam project was divided into three main components (i) to assess the risks of zoonotic disease emergence, (ii) to investigate the environmental and climatic influences on these risks and (iii) to establish preventive strategies to reduce the likelihood of zoonotic disease emergence. In doing so, the project aimed to enhance surveillance systems and move towards a more integrated One Health approach.

The PREZODE-AFRICAM project agreement was signed in October 2024 between the IPC and the Institut pour le Recherche et le Développement. The project involves 4 entities at the IPC: medical

biology laboratory, virology unit, medical and veterinary entomology unit and epidemiology and public health unit.

ECOMORE Project: Strengthening the Health Security in the Indo-Pacific Region

In June 2024, the Agence Française de Développement (AFD) and Institut Pasteur (Paris) signed a €4 million financing agreement as part of the broader €15 million "Strengthening Health Security in the Indo-Pacific Region" program. This four-year initiative aims to enhance epidemic prevention, preparedness, and response (PPR) across member states and territories of the Indian Ocean Commission (IOC), the South Pacific Community (SPC), and the ECOMORE network in Southeast Asia.
Within these three networks, the program will focus on (i) strengthening integrated approaches to human, animal, and environmental health within each network's practices, and (ii) establishing a framework for inter-network cooperation on epidemic prevention preparedness and response.
In Southeast Asia, the activities will be carried out in Cambodia, Laos, the Philippines, and Vietnam, with the involvement of key ECOMORE network partners: IPC, Institut Pasteur du Laos (IPL), Institut de Recherche pour le Développement (IRD), National Institute of Hygiene and Epidemiology (NIHE, Hanoi) and the Research Institute of Tropical Medicine (RITM, Manila)

Risk Assessment of Multiple Endemic Infectious Pathogens in a One Health Perspective (RACSMEI)

From 2025 to 2030, the RACSMEI project aims to enhance Precision Public Health in Cambodia, a high-burden country for infectious diseases. By integrating human, animal, and environmental data through a One Health approach, the project will conduct a nationally-representative survey on several priority zoonotic and endemic diseases, covering 10,000 individuals, animals, vectors, and environmental factors. The research will utilize cutting-edge multiplex serology, environmental sampling, metagenomics and mathematical modeling to understand disease transmission dynamics, focusing on priority pathogens such as dengue, Chikungunya, Zika, Japanese encephalitis, Avian influenza, Nipah virus, Hantavirus, leptospirosis, *Burkholderia pseudomallei*, arenaviruses, tick-borne encephalitis virus (TBEV), and severe fever with thrombocytopenia syndrome virus (SFTSV) and others. The findings will inform targeted interventions, strengthen surveillance, and contribute to informed public health policies and long-term collaborative research.
This project, funded by Wellcome Trust Discovery Award, involves different IPC units, CIRAD, Malaria Consortium, MoH, MAFF.

Characterization of the immunological mechanisms that drive chronic chikungunya disease pathogenesis

This ambitious project (2024-2030), carried out in collaboration with Rockefeller University, and funded by the Wellcome Trust Discovery Award aims to test the hypothesis that dysregulated antibody responses modulate susceptibility to chronic chikungunya through impaired antiviral activity, as well as excessive and inappropriate activation of pro-inflammatory pathways. These studies are expected to lead to the identification of the immune pathways and biomarkers that are associated with disease chronicity, guiding the development of novel vaccination and therapeutic strategies to prevent or treat chikungunya disease.

One Health Regional Approach for Integrated and Interconnected Urban Dengue Surveillance Southeast Asia (SEA-ROADS)

This project "ANRS0637 SEA-ROADS Project: A One Health Regional Approach for Integrated and Interconnected Surveillance of Urban Dengue in Southeast Asia" funded by ANRS MIE addresses three key challenges: (i) understanding the intra-urban spatial distribution of mosquito-borne pathogen risk,

(ii) improving inadequate entomological surveillance, and (iii) enhancing surveillance tools at local, national, and regional levels. A multi-scale approach will integrate human mobility and mosquito habitat suitability under climate change scenarios to predict and mitigate dengue risk.

Pasteur International joint research Unit (PIU) in Immunology and Malaria

In 2024, the agreements organizing 2 PIUs which had been approved in 2023, each integrating an IPC unit (Immunology Unit and Malaria Unit) were signed.

- "Deciphering adaptative immune responses to emerging and epidemic-prone infectious diseases – PIU Immuno" bring together the Immunology Units of IPC, Institut Pasteur de Madagascar and Institut Pasteur de Dakar. The global objective of this proposal is to build a sustainable immunology research ecosystem in the African (Senegal and Madagascar) and South-East Asian (Cambodia) Pasteur Institutes around COVID-19 and dengue research projects.
- The PIU "*Plasmodium vivax* escape strategies to malaria elimination efforts Pasteur International Unit" associating the Malaria Research Unit at IPC with the Plasmodium Genetic Unit at Institut Pasteur de Madagascar and the Infectious Disease Epidemiology and Analytics G5 group at Institut Pasteur Paris) will enable to further understand the challenges revolving around elimination of *P. vivax*.

Creation of a Grant Office (January 2024)

IPC has been managing a large portfolio of grants and is fully dependent on attracting external funds. To enhance pre award and post award support for IPC'scientists and to reduce the administrative burden for scientists and ensure full compliance with a diverse range of granting bodies, a new structure called the "Grants Office" (GO) was established in 2024.

Mrs. Najet Hadhri was recruited as Grants Officer, starting remotely in January 2024 and moving to on-site work in July 2024. The GO has been also reinforced with the recruitment of an additional resource, prioritizing post-award qualifications to ensure effective management of IPC projects portfolio.

CESRI at IPC, November 5th-8th.

The IPC hosted the meeting of the Committee for the Evaluation of Scientists of the International Network (CESRI) from 5 to 8 November 2024. This committee meets annually to evaluate the national scientific staff with the status of 'International Network Scientists' of six Pasteur Network member institutes: the CERMES in Niger, the Centre Pasteur in Cameroon, the Institut Pasteur de Bangui, the Institut Pasteur du Cambodge, the Institut Pasteur de Dakar and the Institut Pasteur de Madagascar. Over the course of the four days, the committee assessed career advancements, including promotions and the integration of more than 70 scientific personnel.

Training and knowledge dissemination

6th International Conference on the Tiger Mosquito, *Aedes albopictus,* March 28-29th

Dr Sébastien BOYER and the Medical and Veterinary Entomology Unit organized the 6th International Conference on the Tiger Mosquito, *Aedes albopictus.* The Conference has been opened by H.E. Prof. CHHEANG Ra, Minister of Health of the Kingdom of Cambodia, and H.E. Jacques Pellet, the French Ambassador in Cambodia. In the absence of a vaccine strategy, the fight against vector species is decisive, requiring a good knowledge of the vector.

The conference was therefore an important opportunity to share knowledge about *Ae. albopictus*. Internationally recognized scientists from 21 different countries were able to exchange knowledge

and new discoveries, participate in discussions, develop new, innovative and adapted strategies, and strive for quality collaborations. Organized every two years on an international scale, this conference has already been held in China (Guangzhou, twice), Italy (Pavia, twice) and France. This is the first time the event has been held in Southeast Asia, in Cambodia to be precise.

Workshop on Biosecurity Risk Management, July 23-26th

In July, the Institut Pasteur du Cambodge (IPC) and the National Authority of Chemical Weapons (NACW) co-hosted a capacity-building workshop on Biosecurity Risk Management. This workshop, supported by the European Union's CBRN Centres of Excellence Project 81 - BIOSEC Enhanced Biosecurity in South East Asia, aimed to strengthen regional capacities in managing biological risks across environmental, human, and animal health sectors, focusing on laboratory activities.
Over 40 participants from various One Health sectors engaged in hands-on exercises at IPC, mastering biological risk assessment and advanced methodologies, while aligning with international standards such as the WHO Laboratory Biosafety Manual (4th Ed).

The Avian Influenza Symposium, Phnom Penh, December 10-12th

Opening by His Excellency Prof. KOY Vanny, Secretary of State, Representative of H.E. Prof. CHHEANG Ra, Minister of Health, this event, organized by the Pasteur Network and the IPC, brought together experts to discuss challenges of avian influenza. Experts from the Asia-Pacific region presented their One Health approach, offering lessons learned and a vision for more integrated future responses
This symposium was organized by Dr Erik KARLSSON (IPC) and Pr Vijaykrishna Dhanasekaran (IP Hong-Kong). These presentations underscored the Pasteur Network's coordinated, multifaceted efforts in combating avian influenza, focusing on surveillance, prevention, and regional collaboration.

The 3rd RAPID Symposium on Antimicrobial Resistance in IPC, December 11th

On December 11th, 2024, the IPC hosted the 3rd RAPID Symposium under the theme "One Health Perspective to Address antimicrobial resistance (AMR) in the Asia-Pacific Region. The event brought together experts, researchers, and policymakers to address the pressing issue of antimicrobial resistance (AMR). Discussions focused on the AMR research landscape in Cambodia and other countries from Asia- Pacific region, antimicrobial stewardship, genomic tracking, and the effects of climate change on resistance. Speakers from the health, agriculture, and scientific communities presented findings and insights, fostering collaboration and advancing efforts to combat AMR in the region.

Scientific Writing Training at IPC, May 6-10th

From May 6 to 10, 2024, the IPC organized a 7-hour day training in scientific writing. Fifteen scientists from 7 different units and laboratories benefited from this important training delivered by EPCONCEPT trainers. The objectives of this course based on practical exercises were: (i) to prepare an argument matrix for a scientific article, (ii) to prepare tables and figures fitting and describing the results of an investigation, (iii) to develop a detailed outline of the manuscript, (iv) to write all manuscript chapters and paragraphs based on the scientific matrix, detailed outline, chosen tables and figures.
This was the first course of its kind conducted at the IPC.

Three IPC Students defended their PhD in 2024

Three IPC students defended their PhD in 2024: Mrs. IV Sophea, Epidemiology and Public Health Unit (Paul Sabatier-Toulouse III University, France), Mr. David GUERRERO GOMEZ, Immunology Unit (University of Montpellier, France and, Mrs. SANN Sotheary, Immunology Unit (University of Hasselt, Belgium).

UHS–IPC Scientific Seminars

In 2023, the University of Health Sciences (UHS) and the IPC launched an initiative to maintain a series of scientific seminars involving researchers from both institutions to promote information sharing, interactions, collaborations.

In 2024, 4 sessions have been organized (risk on zoonotic disease from swine production, evaluation of T cell responses to rabies post-exposure prophylaxis, comparison of 2 strategy to improve detection of active HCV infection in Cambodia, impact of systematic TB detection on mortality in children aged under 5 with severe pneumonia).

Publication of the book on "the Insects in Cambodia" in Khmer, French and English"

The Medical and Veterinary Entomology Unit published a book to the destination of the children and confirms its commitment to civil society. Our team realized an educational scientific project, for everyone, especially aimed at young people.

As a result, the captivating book entitled "Insects of Cambodia" was published in January 2024. This book, published in three languages (Khmer, French, English), is primarily intended for young minds aged 8 to 12.

Public Health

Providing Expertise to the Ministry of Health Regarding Emerging Diseases

The IPC's three WHO reference centres carried out public health activities alongside and in support of MoH teams, including in 2024: (i) monitoring human and avian influenza viruses (14 human cases of highly pathogenic avian influenza virus H5N1), and (ii) the virological confirmation of 18 cases of monkeypox virus infection.

Inauguration of the new Kampong Cham Rabies Prevention Centre (May, 6th)

On Monday, 6th May 2024, the IPC inaugurated with the Ministry of Health our new Rabies Prevention Center in Kampong Cham. This new IPC-KC PHD Rabies Prevention Center replaces the previous one installed in 2019. The ceremony was held under the presence of HE. Dr. Ngov Kang, Secretary of State, MoH, HE. HAN Kosal, the Deputy Provincial Governor of the Kampong Cham Province, the Directors and Deputy Directors of the Provincial Health Department (PHD), and the IPC directions.

The new center is a single storey building of 180 m^2 (12 meters wide by 15 meters long), it's designed based on IPC teams' experience acquired over several years in this expertise. The center is more visible and convenient for IPC to provide PEP and vaccination services in the best reception and safety conditions.

Health services

Quality: accreditation of Medical Biology Lab. and Lab. for Environment and Food Safety

In 2018, the MBL obtained ISO 15189 accreditation for biochemistry, haematology, and microbiology. In 2022, renewal of this accreditation by the French Committee for Accreditation (COFRAC) was granted for a period of 5 years. In 2024 our laboratory completed the transition to ISO 15189:2022 standard, which replaces ISO 15189:2012. The aim of this revision is to improve patient care and user satisfaction in medical laboratories through confidence in quality and competence.

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. In 2024, LEFS broadened its accreditation scope by adding five more parameters to its five

existing food microbiology parameters, along with four additional parameters in water microbiology, as detailed below.

CAPRED project: heavy metal testing, pesticide and antibiotic residue testing.

In 2024, the received financial support from the Cambodia Australia Partnership for Resilient Economic Development (CAPRED) to establish new services for heavy metal testing, as well as pesticide and antibiotic residue testing. The objective is to establish accredited laboratory services with ISO/IEC 17025:2017 for Heavy Metal, Pesticide and Antibiotic testing to promote food safety and export. It is a 2.5-year project from April 2024 to Dec 2026.

For pesticide residues analysis, the liquid chromatography couple with mass spectrometry (LC-MS/MS) and gas chromatography couple with mass spectrometry (GC-MS/MS) were installed in September 2024. For Heavy Metal analysis, the Graphite Furnace Atomic Absorption Spectrometry (GFAAS)was completely set up by the end of March 2024.

A new Laboratory Information System for the Medical Biology Laboratory

At the end of 2023, a new Laboratory Information System (LIS) was implemented at the MBL, DGLab, in December 2023. In 2024, the LIS had to be regularly reviewed, updated, and improved to ensure its suitability for the MBL's needs. Improvements to the LIS will continue in 2025.

In December 2024, MBL implemented DGWeb, a web application designed to provide healthcare professionals with simple and secure access to the reports generated by our DGLab LIS.

Management

IPC 2030 : a major campus restructuring project

IPC has launched IPC 2030, an ambitious project to restructure and modernize its campus, including the construction of a new building to support future growth. The first phase of the study (initial programming) has been completed, defining the key organizational and structural aspects of the project. At the end of this phase, the total investment was estimated at approximately $20 million.

The project was presented to the Institut Pasteur in Paris (IPP) in April 2024 and at the Liaison Council meeting on 24 May 2024, and was very well received by both the IPP and the Ministry of Health of the Kingdom of Cambodia. Currently, initial discussions with potential funders are underway, in coordination with Institut Pasteur Paris, and MoH to explore financing options and ensure the project's successful implementation.

Modernization of finance and human resources functions

As part of the modernization of IPC's support functions, the CFO's services modernized their financial and human resources functions by developing the NOTIC software package and relying on a consulting mission (Korn Ferry). NOTIC is a new management and accounting system that marks a major step forward in streamlining financial operations and improving overall efficiency by giving scientists greater visibility and autonomy in carrying out certain expenses.

In 2024, IPC launched a comprehensive job mapping initiative to establish a clear and structured job classification system. This job mapping exercise serves as a foundation for future HR policies, including career progression, compensation benchmarking, and talent management strategies. It ensures that IPC remains competitive in attracting and retaining talent while aligning with international best practices.

In Conclusion

The year 2024 was marked by the acquisition of major projects driven by the dynamism of the Institute's scientists (Welcome Trust, NIH, AFD, BMGF, etc.). This dynamism also resulted in the creation of two entities: a new research unit focused on bioinformatics and artificial intelligence, and a Grant Office aimed at reducing the administrative workload of researchers for project management during calls for proposals. Efforts to train future scientists (PhD students and young scientists) continued, reaching an unprecedented level (17 PhD students during 2024).

However, there are some worrying elements. The IPC had to cope with unplanned departures from the Virology Unit, including its unit head, which makes the situation in this sensitive unit somewhat worrying and requires the attention of the management of this unit and the Institute.
Moreover, the IPC's economic model that is still based on revenues and on the mobilization of external donors, must be closely monitored. More than ever, our Institute needs the commitment of the Institut Pasteur in Paris and of the Royal Government of Cambodia to develop.

Finally, I would like to thank all our staff for their efforts and commitment to delivering excellent work and enabling our Institute to fulfil its missions for the benefit of the people of Cambodia.
I extend my warmest thanks to the Scientific Council, which will meet in 2025, and to the members of the Liaison Council, particularly its President, H.E. Prof. CHHEANG Ra, Minister of Health, for their guidance and support to our Institute.

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

2 Institut Pasteur du Cambodge in 2024

2.1 Overview of the Institute

Statutes and Operational Systems

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

Governance

The IPC is led by a director and is monitored by the Liaison Council. The IPC's director is appointed by the President of the Institut Pasteur in Paris, France, in consultation with the Kingdom of Cambodia's MoH. The IPC's deputy director is nominated by the director, in consultation with the MoH, from within the pool of Cambodian scientists serving in the national public service and who have doctorates in biology or public health.

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

The IPC's scientific activities are also reviewed every two or three years by the Scientific Advisory Board, whose last session was held in early February 2021. In 2023, the composition of the Scientific Advisory Board was modified by the appointment of new representatives from the MoH. For reasons of availability of SAB members, it was not possible to convene a meeting of the Council in 2024. The next meeting is scheduled for 26-28 March 2025. Our scientific strategy is then adapted, based on the recommendations from both the Liaison Council and the Scientific Advisory Board.

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

2.2 Structure

The organizational chart appears at the end of this report, in Annex, section 8.2, page 205.

The Institute is composed of:

- A management unit comprising the director, the deputy director, and the chief financial officer;
- Administrative, financial and logistical services;
- Six research units: Malaria, Epidemiology and Public Health, Immunology, Medical and Veterinary Entomology, Virology, Bioinformatics and AI Applications;

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

2.3 Human Resources

As of 31 December 2024, the Institute had a team of 298 personnel of 17 different nationalities, with 88 % being Cambodian nationals:

- 288 with IPC contracts (55 of which are civil servants attached to the MoH);
- 9 Cambodian scientists (1 research director, 7 research fellows, and 1 research assistant);
- 9 Cambodian PhD students;
- 37 expatriates, including:
    - 10 on institutional contracts: 9 from Institut Pasteur in Paris (IPP), 1 Expertise France, and
    - 27 on IPC contracts, including 2 scientific seniors, 1 IT engineer, 12 postdocs, 6 PhD Students, and 7 others (project managers,…).

Diversity and Leadership

The Institute prioritizes gender balance and equity; 58 % of staff members are women. The professional development of scientific leaders and other national professionals is a matter of great importance to the IPC. Of the 19 management positions (3 directors, 9 research or service unit heads 7 support services managers), 5 are occupied by women (24 %) and 7 (34 %) by Cambodian nationals.

2.4 Finances and investments

2.4.1 Finances

The majority of IPC's revenue comes from binding research contracts funded by donors (29%), from services offered by IPC (57%)—including those for rabies prevention and treatment—and from a subsidy provided by the Ministère de l'Enseignement Supérieur, de la Recherche, et de l'Innovation (the French Ministry for Higher Education, Research, and Innovation, MESRI) via the Institut Pasteur in Paris (table 1).

The Royal Government of Cambodia does not directly fund IPC, but it makes a significant contribution through tax and customs exemptions. Table 1 provides details on the different revenue streams, while Figures 1 and 2 below illustrate the breakdown of funding by country and by donor in 2024.

	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Research contracts	53%	60%	58%	59%	56%	56%	54%	40%	22%	28%	34%	**29%**
Health services	26%	21%	23%	25%	29%	31%	34%	51%	71%	47%	48%	**57%**
MESRI grant	17%	14%	12%	11%	11%	11%	10%	6%	3%	5%	4%	**5%**
Other revenues	5%	5%	7%	5%	5%	2%	2%	2%	4%	20%	14%	**9%**
	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	**100%**

***Table 1**. Revenue breakdown by source type (2013–2024)*

The share of income attributable to service activities primarily comes from our International Vaccination Unit, Medical Biology Laboratory, and Consulting/Expertise in Public Health. Other revenues mainly stem from financial investments.
The bad debt related to COVID-19 invoices was settled at the beginning of 2025 with the support of the Ministry of Health and the Ministry of Economy and Finance.

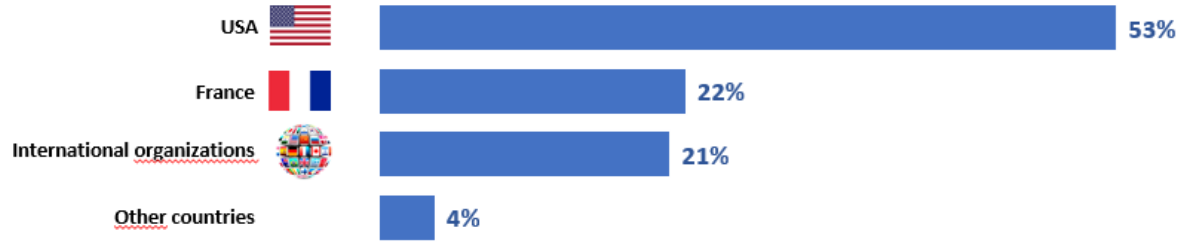


Figure 1. *Breakdown of the research funds received by country*

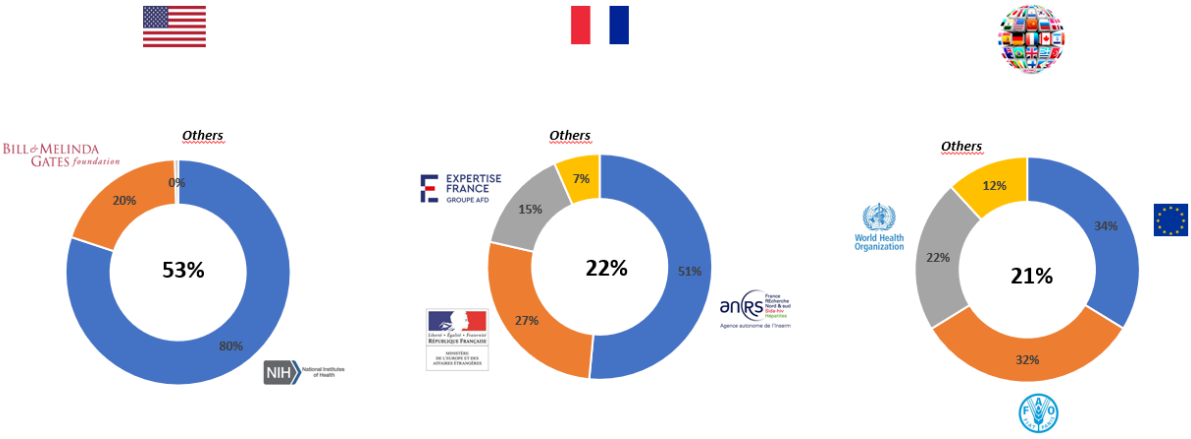


Figure 2. *Breakdown of the research funds received by donor*

2.4.2 Equipment Purchases

Total equipment purchases exceeded $1,740,592 in 2024, more than 52 % of which were financed by IPC's own funds.
The main purchases are as follows:

- New analytical equipment for LEFS, with a total investment of $748,856, 80% of which was financed by Australia's Department of Foreign Affairs and Trade under the CAPRED program,
- Implementation of the DG Lab System ($297,678), a Laboratory Information Management System (LIMS) designed to enhance the efficiency and traceability of service laboratories, fully financed by IPC's own funds,
- Deployment of Notic ($126,800), the new integrated system for accounting, order tracking, and financial control, fully financed by IPC's own funds,
- Construction of the new anti-rabies center building in Kampong Cham ($124,000), fully financed by IPC's own funds,
- Acquisition of a new autoclave ($62,373), donated by the European Union.

2.4.3 Implementation of Notic: A New Management and Accounting System

As part of its modernization efforts, IPC has implemented Notic, a new management system developed on Dynamics 365. This transition marks a major step forward in streamlining financial operations and improving overall efficiency.

The implementation of Notic also introduced two significant changes:

- A change in accounting currency, with IPC's financial records now maintained in US Dollars instead of Euros. This adjustment aligns IPC's accounting practices with its operational and financial environment.
- A transition to IFRS for SMEs, replacing the previous accounting standards. This shift enhances financial reporting transparency and ensures compliance with internationally recognized best practices.

2.4.4 Implementation of a Job Mapping Framework

In 2024, IPC launched a comprehensive job mapping initiative to establish a clear and structured job classification system. By defining roles and responsibilities more precisely, the initiative enhances internal consistency, career development opportunities, and overall transparency in job positioning.

This job mapping exercise serves as a foundation for future HR policies, including career progression, compensation benchmarking, and talent management strategies. It ensures that IPC remains competitive in attracting and retaining talent while aligning with international best practices.

2.4.5 IPC 2030: A Major Campus Restructuring Campus

IPC has launched IPC 2030, an ambitious project to restructure and modernize its campus, including the construction of a new building to support future growth. The first phase of the study (initial programming) has been completed, defining the key organizational and structural aspects of the project. At the end of this phase, the total investment was estimated at approximately $20 million.

Currently, initial discussions with potential funders are underway, in coordination with Institut Pasteur Paris, to explore financing options and ensure the project's successful implementation.

2.5 Publications in 2024

The IPC's research and public health activities are detailed in later sections (section 8, page 188). A summary of these is presented in figures 3 and 4 below.

Research activities done in 2024 were featured in 60 articles published by scientists affiliated to IPC, appearing in peer-reviewed international journals. Among them are 30 (50%) as first or last author and 35 (58%) with an IF greater than or equal to 4 (figures 3 & 4).

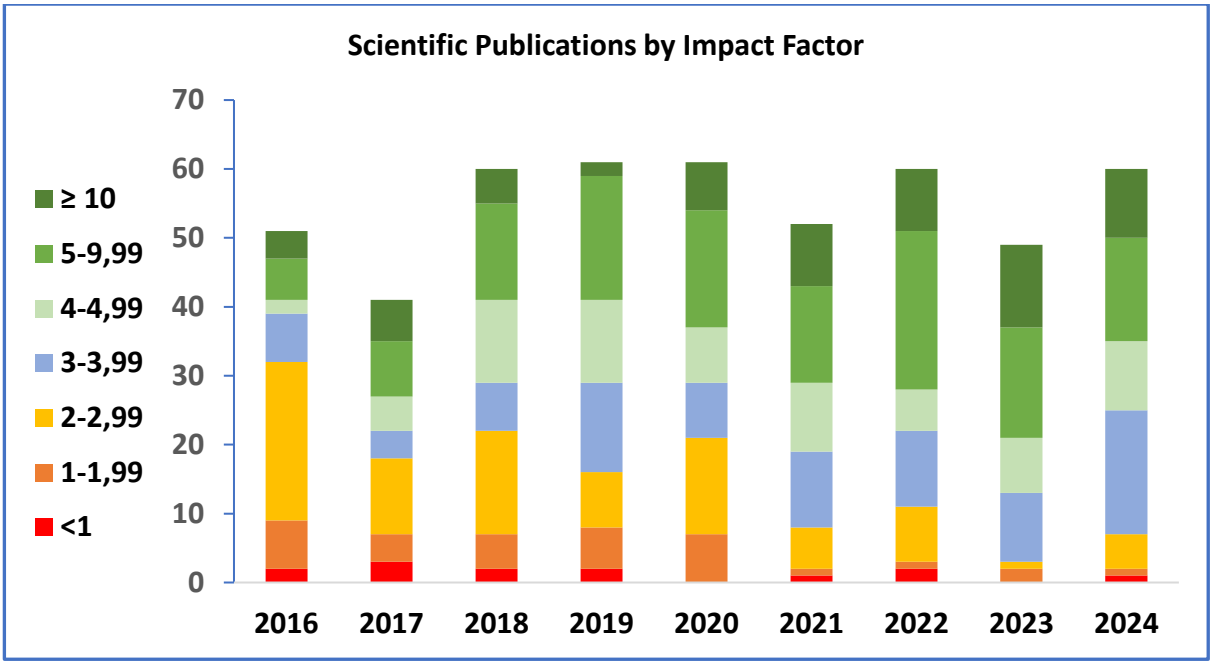


Figure 3: *Scientific publications, sorted by impact factor of the journal (2016-2024)*

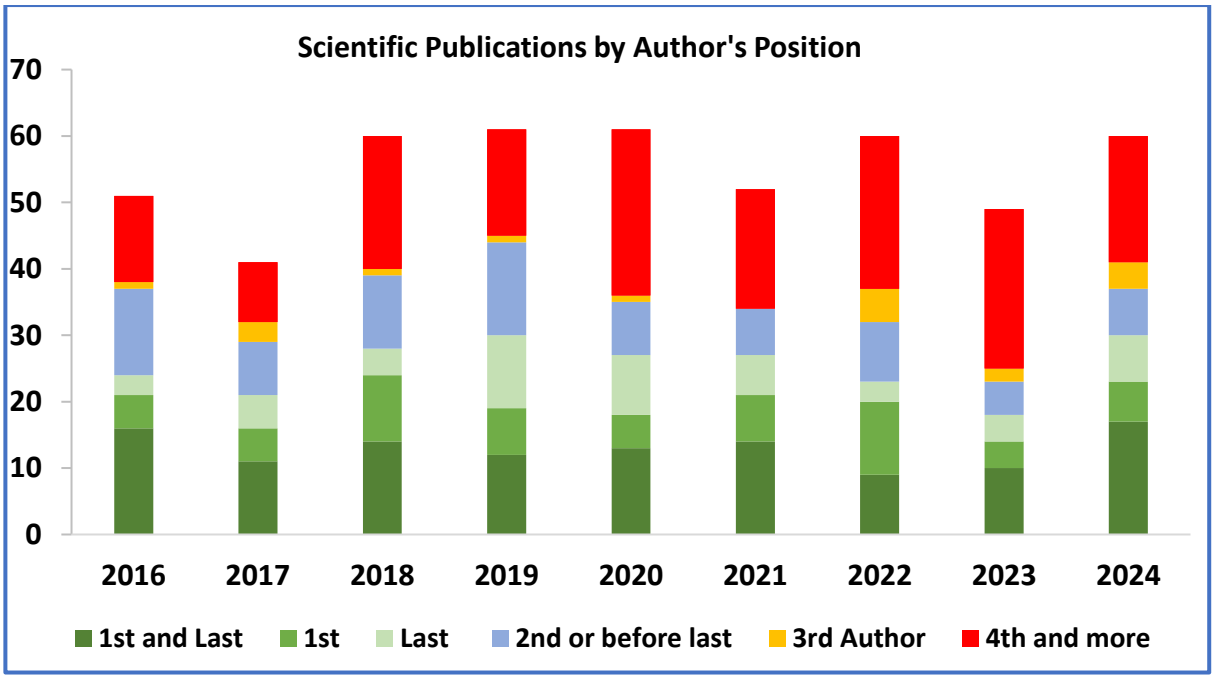


Figure 4: *Scientific publications by author's position (2016-2024)*

2.6 Training and Internships in 2024

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

Student Internships

Throughout 2024, 154 students interned at the IPC. This is higher than the 131 welcomed in 2023. Among the 154 students, 128 were Cambodian nationals. Their university affiliations are as follows: University of Health Sciences (86), University of Puthisastra (12), the Institute of Technology of Cambodia (6), the Royal University of Phnom Penh (3) while the remainder (50) were from various French and Belgian universities. Seventeen were PhD students (11 Cambodian of whom 2 defend their PhD in 2024), 26 were in master's level studies, 87 were bachelor-level students, and 14 were working toward associates' degrees.

International Master's in Infectious Disease

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

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

The 2024-2025 academic year welcomes 16 students (9 men and 7 women) of 7 nationalities, including 4 Cambodians (3 M1 students and 1 M2 student).

As of 2024, a total of 69 students of 16 different nationalities were admitted to this International Master's Program.

	M1	M2
2019-2020	**P1:** 9 students (4 Cambodians)	
2020-2021	**P2**: 9 students (4 Cambodians)	**P1**: 9 students (4 Cambodians)
2021-2022	**P3**: 7 students (2 Cambodians)	**P2:** 9 students (4 Cambodian) + 4 new students
2022-2023	**P4**: 14 students (4 Cambodians)	**P3**: 9 students (3 Cambodians) + 4 new students
2023-2024	*Class was not open due to insufficient students' registrations*	**P4:** 12 students (4 Cambodians) + 7 new students
2024-2025	**P5**: 8 students (3 Cambodians)	**P5**: 8 new students (1 Cambodian)

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

2.7 Scientific Seminars

The IPC held scientific seminars every other week. 27 were held in 2024 (table 3)

Date	Names Surnames	Grade	Organization / Units	Title
10-Jan	Chainoy SOEM	Deputy Head	LEFS	Activities of Laboratory of Environment and Food safety
24-Jan	Thavry HOEM	PhD Student	Virology	Population Dynamic and Spatial Distribution of Rhinolophus sp. bats: Contribution to Risk Assessment of Coronaviruses Spillover in Cambodia.
5-Feb	Claire GUINAT	Research Scientist	INRAE	Understanding the transmission dynamics of avian influenza viruses - past and future projects
6-Feb	Arnaud FONTANET	Professor	Institut Pasteur Paris, France	Impact of environmental and climatic changes on infectious diseases emergence
28-Feb	Quique BASSAT	Researcher	Barcelona Institute for Global Health	Innovation and Global health in pediatrics: From good ideas to impactful ones
6-Mar	Vinit UPASANI	Post-doc	Immunology	Immune responses to SARS-CoV-2 in immunocompromised hosts
20-Mar	Neil FUREY	Researcher	Independent researcher	Another leap in the dark: the utility of bioacoustics for surveilling bat populations in diverse environments in SE Asia
26-Mar	Stephen M TOMPKINS	Researcher	University of Georgia, USA	Influenza B: the other influenza virus
27-Mar	Didier FONTENILLE	Researcher	IRD	Mosquito vs Sapiens: guilty or not guilty? Revisiting human – vector relationships in a one health approach.
10-Apr	Antsa RAKOTONIRINA	Post-doc	Entomology	Exploring the Techniques of Vector Identification and Pathogen Detection in Medical Entomology
24-Apr	Dorothée MISSÉ	Researcher	IRD	Deciphering arbovirus entry and infection mechanisms for pathogenesis understanding and therapeutic target identification
29-Apr	Etienne-SIMON-LORRIÈRE	Researcher	Institut Pasteur, Paris, France	Leveraging viral genomic data for outbreak response
15-May	Sanary KAING	Researcher	EpiPH	TB-Speed: Experiences and Perceptions of Health Care Workers (HCWs) in decentralizing childhood TB diagnosis
29-May	Sony YEAN	PhD student	Entomology	Seasonal dynamic of ticks infesting cattle (Bos indicus) farms in Kampong Speu and Takeo province, Cambodia

5-Jun	Malen CHAN	Researcher	EpiPH	Potential Impact of a Social Media Event on Rabies Post-Exposure Prophylaxis (PEP) in 2019 at Institut Pasteur du Cambodge (IPC), Cambodia
19-Jun	Linda SOV	Researcher	EpiPH	The Prevalence of Low Birth Weight and Its Associated Risk Factors among Hepatitis B Infected Pregnant Women in Cambodia: Analysis from the ANRS 12345 TA PROHM Study
3-Jul	Jolein LAUMEN	Post-doc	G4-AMR	Untreatable gonorrhoea: are we out of time?
17-Jul	Brice FEUFACK DONFACK	Post-doc	Malaria	Invasion of Plasmodium vivax into human reticulocytes and response to antibodies
23-Aug	Etienne JACOTOT	Researcher	Inserm	Selective Caspase-2 Inhibition in Alzheimer's Disease
28-Aug	Josephine JUET	M2 Student	EpiPH	Drug consumption in Cambodian communities (Takeo), spotlight on interactions between local people and antimicrobials
11-Sep	Candice BOHAUD	Post-doc	Immunology	The Characterization of DENV E-Dimer-Specific Memory B Cells and Antibodies
9-Oct	Herman BANZA KONGOLO	PhD Student	EpiPH	25 years of rabies post-exposure prophylaxis in Cambodia (1998 to 2022) and vaccination compliance
18-Oct	Bastien MALINGE	PhD Student	INRAE	Identifying environmental viral shedding of Coronavirus, Astrovirus and Hantavirus by the Jamaican fruit bat in Mexico to assess the value of fresh fecal sampling for zoonosis surveillance
4-Nov	Julien PETILLON	Professor	Entomology	Drivers of (neo) tropical spider diversity
20-Nov	Vouchleang SRENG	Researcher	EpiPH	Optimizing Tuberculosis Preventive Treatment Initiation Among People with HIV in Cambodia: Results of the OPTICAM Cluster randomized Trial
4-Dec	Sokha CHEA	PhD student	EpiPH	Wildlife Health Surveillance Network Lumpy Skin Disease Detection & Response in Cambodia
18-Dec	Bunnet DIM	Deputy Head	EpiPH	The Preliminary Result of the Third TB National Prevalence Survey 2023-2024

*M1 = Master 1, *M2 = Master 2

Table 3: List of the 27 seminars held in 2024

2.8 Visits and missions at IPC in 2024

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

Date	Visits and missions
26-Jan	H.E Prof. CHHEANG Ra and MoH's Delegation, Minister of Health
05-07-Feb	Prof. Arnaud FONTANET, Head of Epidemiology of Emerging Diseases, Insititut Pasteur in Paris
8-Feb	Cambodia Academy of Digital Technology (CADT)'s Delegation, led by Dr. Didier GUY, Expertise France, Senior advisor for entrepreneurship & digital innovation with the Ministry of Post and Telecommunications of Cambodia
25-29-March	Dr. Didier FONTENILLE, Research director emeritus, Institut de Recherche pour le Développement (IRD) and former director of IPC
28-Mar	Prof. S. Mark TOMPKINS, Ph.D., Director of Center for Influenza Disease & Emergence Research and Center for Vaccines and Immunology, University of Georgia, USA
6-May	Join External Evaluation (JEE) team led by Dr. Cui LIN, Co-Chair of International Evaluation Expert Group
16-May	CIRAD delegation led by Dr Martijn TEN HOOPEN, Vice Director of the Biological Systems Department (BIOS)
17-May	IPC 2030 project meeting with AFD, Mr. GONNET Thomas and Mr. DOLLFUS Emmanuel
20-May	AFD delegation led by Ms. Marie-Hélène LOISON, Deputy General Director AFD
21-26 May	Mrs. Mathilde BOISSERIN, Regional Advisor Pacific-Asia, International Affair Department, Institut Pasteur in Paris
23-25 May	Dr. Vincent RICHARD, International Affair Department, Institut Pasteur in Paris
28-May	National Authority of Chemical Weapons delegation led by H.E General PHORN Nara, the Secretary General for the CNBRW
29-Jul	André FURCO, Health Advisor, French Ministry of Foreign Affairs and International Development
30-Jul	Mrs. Juliette PERROT, Global Health regional program officer, French Embassy in Thailand
22-Aug	US congressional delegation and partner led by Mr. Brad WENSTRUP, Member of the U.S. House of Representatives
18-Sep	Mr. Luc LE CALVEZ, Representative of Institut de Recherche pour le Développement (IRD) in Cambodia
23-Sep	Ms. Sylvie GUILLEMAUT, Financial Coordinator at the International Affairs Department of Institut Pasteur in Paris, ECOMORE3 Project
26-Sep	WHO delegation, led by Dr Jaana Marianna TRIAS, WHO representative in Cambodia
7-Oct	Dr. Didier LAUREILLARD, DATURA coordinating investigator and Dr. Olivier SEGERAL, the HEPEDIAC and HIPOCAMP coordinating investigator

7-Oct	Prof. Françoise BARRE-SINOUSSI, ANRS, IPP
11-Oct	Dr Daniel NGAMIJE, Director of the Global Malaria Programme, WHO in Geneva
24-Oct	Dr Rosalina SA AGA-BANUVE and Dr Esabelle YAM from National Centre for Epidemiology and Population Health of Australian National University
25-Oct	Mrs. Marie ENGEL, Director of International Operations and Laboratory, Fondation Mérieux from Lyon
30-Oct	H.E. Igor DRIESMANS, Ambassador of the European Union (EU)
20-Nov	Mr. Daniel SCOTT-ALGARA, Scientific General Secretary at the Institut Pasteur in Paris
2-Dec	CIRAD delegation, led by Mr. Denis GAUTIER, Deputy Director, Environments and Societies Department
4-Dec	Prof. Lulla OPATOWSKI, Université de Versailles Saint Quentin (UVSQ) / Inserm / Institut Pasteur, Institut Pasteur unit "Epidemiology and Modelling of Antibacterial Evasion"
4-Dec	KFW in collaborate with German Embassy, led by Ms. Christina LAUN, Head of Division for South-East Asia
12-Dec	Sung Key JANG, CEO of Institut Pasteur in Korea, Prof. Leo Poon, Co-Director of HKU-Pasteur, scientists and experts from the Pasteur Network, visit IPC as part of the Avian Influenza Symposium.

Table 4: *Visits, missions and delegations received at the IPC in 2024*

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

The general objectives remain the same as those of previous years, in line with recommendations made at the last two Liaison Councils.

2.9.1 Defining our Scientific Strategy

Organizing the 8th Meeting of the SAB

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

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

Developing a 5-year Strategic Plan

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

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

Discussions and exchanges with IPC scientists on this topic began in November 2024. Meetings were scheduled with our main partners at the MoH and MAFF during the first quarter of 2025 to gather

their views on (i) the IPC's place in Cambodia's health and research systems, and (ii) the nature of our future partnerships.
The plan should be finalized in 2025 and should demonstrate how the IPC can build local scientific capacity and skills transfer to local health authorities and actors. This would allow the IPC to concentrate on highly specialized public health issues as well as on research.

2.9.2 Continuing the Development of Quality Standards

The IPC currently has three accredited laboratories (MBL, LEFS, and the Metrology Laboratory).
The Virology Unit, due to its size and to the critical activities it carries out (diagnoses critical for public health), has begun developing a quality approach in 2023.
Finally, a certification process (ISO 35001:2019) was initiated in 2023 for our BSL-3 laboratory as part of our commitment to continuous improvement in biosafety. Achieving this ISO 35001:2019 certification is anticipated for 2025.

2.9.3 Promoting Staff's Career Development & Making IPC a More Attractive Workplace

Within 5 to 10 years, Cambodian scientists should make up at least 75% of the scientific staff and more than 50% of the units should be headed by Cambodians. This will involve identifying young talent, training them and, for the most promising, building career paths that are attractive enough to motivate them to stay at the Institute. The criteria for identifying these future elites among the young talents must include an assessment of managerial potential in the same way as 'intellectual and technical' potential.

Supporting PhD Students

The IPC shall continue to support the training of young Cambodian scientists by covering the costs related to their PhD. In 2024, 9 PhD Students were supported, for a total of around €100,000.

Strengthening Management Skills

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

Professional Development and Career Attractiveness

In order to identify "young talents" with high potential within the Institute and to plan career paths, the IPC organizes meetings with each head of unit, together with the Human Resources department and management (director, deputy director, CFO). These "Young Talents" meetings began in 2022 and will continue ever since. The process makes it possible to identify young talents (promising laboratory technicians, young PhD students, etc.) and to determine paths allowing their personal development in line with the Institute's strategy).

Improve Workplace Safety and Security

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

2.9.4 Improving the IPC's Infrastructure: Project IPC 2030.

The IPC project must be presented to the donors. The year 2025 is crucial for assessing our ability to raise the funds needed to finance this important project. The support of the MoH and the Institut Pasteur is fundamental to this process.

2.9.5 Fine-tuning the IPC's Economic Model

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

The Medical Biology Laboratory's activities need to be expanded to generate additional revenue. This is possible, given the technical excellence and reputation it possesses. The laboratory's activity level, measured in "B" according to the French nomenclature of biological acts, which quantifies the activity, is too modest in view of the excellence of its technical platform and its potential to allow even more attractive prices. This laboratory is facing increasing competition from non-accredited laboratories and suffers from accessibility constraints due to a lack of parking space for patients. The establishment of an off-site sampling centre warrants prompt consideration, as does an overall review of the MBL's general positioning in terms of price, value, and access. This project has been relaunched with the arrival of the new MBL's manager in January 2023. An extension to opening hours, which should have taken place in 2024, has been delayed. A project for an external sampling centre is also being studied.

For the LEFS, in 2023 and 2024, significant investment was made with the support of CAPRED (the Cambodia–Australia Partnership for Resilient Economic Development to increase the LEFS' capabilities to heavy metal, pesticide and antibiotic residues analysis capabilities.
This new analysis panel will have to be accredited so that the LEFS can carry out the analyses required for the export of foodstuffs requiring this certification. This new capacity will help the economic development of these sectors in Cambodia and contribute to the IPC's financial autonomy.

3 Research and Public Health Activities

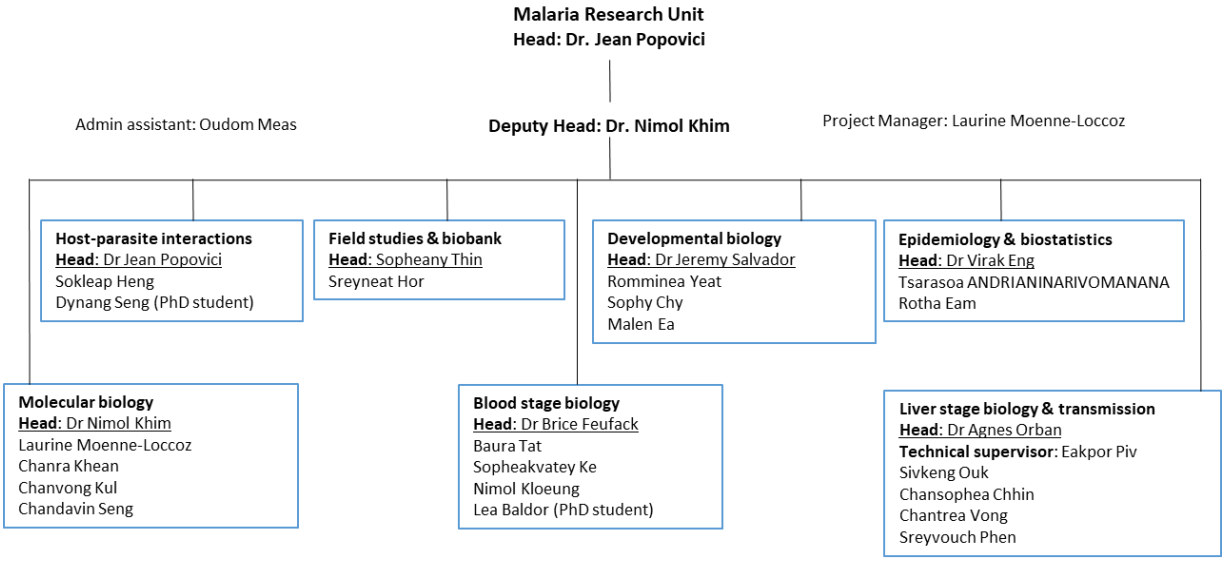
3.1 Malaria Research Unit

3.1.1 Functional Structure

The Malaria Research Unit (MRU) is led by Jean POPOVICI since September 2022. The Unit encompasses six thematic areas, each led by one of the unit members: epidemiology & biostatistics, molecular biology, liver-stage biology & transmission, blood-stage biology, developmental biology and host-parasite interactions.

As of March 2025, The Unit is composed of one Head of Unit (J. POPOVICI–IP permanent researcher), one Deputy Head (KHIM Nimol–IPC permanent researcher), four postdoc researchers (Brice FEUFACK, Agnes ORBAN, Jeremy SALVADOR and Tsarasoa ANDRIANINARIVOMANANA), one junior research assistant (ENG Virak), two PhD students (SENG Dynang and Lea Baldor), one field studies and biobank supervisor (THIN Sopheany), five research engineers (HENG Sokleap, Laurine Moenne-Loccoz, TAT Baura, OUK Sivkeng and YEAT Romminea) and fifteen technical & administrative staff. The MRU is the WHO's main Southeast Asian laboratory for molecular surveillance of malaria diagnostic and drug resistance and, since January 2023, is a referral laboratory for the WHO Malaria NAAT EQA (Nucleic Acid Amplification Testing External Quality Assurance) scheme.

In 2023, a Pasteur International Unit (PIU) has been created associating the MRU at IPC with the Plasmodium Genetic Unit at Institut Pasteur de Madagascar (Benoit Witkowski) and the Infectious Disease Epidemiology and Analytics G5 group at Institut Pasteur Paris (Michael White). This PIU will enable to further understand the challenges revolving around elimination of *P. vivax*. The MRU is also member of the Asia-Pacific International Center of Excellence in Malaria Research (ICEMR) aiming at understanding the epidemiology of malaria in Cambodia and in Papua-New Guinea and of the VISPA consortium aiming at advancing serological diagnostic to provide radical cure treatment.



3.1.2 Research Programs – Major Achievements in 2024

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) used to be a major activity of the unit. In the past years this has been reduced in terms of commitment and now mainly concerns support to other countries. This program is supported by the WHO.

In the past years, samples of *P. falciparum* were collected during therapeutic efficacy studies (TES) performed by Malaria Control Programs in each country of the region. However, with the numbers of cases decreasing, Cambodia has replaced traditional TES by integrated drug efficacy surveillance (iDES) where follow-up of infected patients is done continuously.

We are the main laboratory for characterizing resistance profiles of *P. falciparum* collected in these TES or iDES. The main result obtained from 2024 is the characterization of lumefantrine resistant parasites from Laos (**Feufack et al, in preparation**).

We have also established a collaboration with Jerome Clain (unite MERIT, University Paris Cite and CNR Paludisme) to investigate pyrimethamine resistance in Plasmodium ovale (**Joste et al, 2024, The Lancet Microbe**).

Research Project Name	MalaWHO
Funding	WHO
Project duration	2024 (annual contract)
External collaborator	National Malaria Control Programs (NMCPs): Cambodia, Vietnam, Laos, and WHO (Pascal Ringwald)

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. We have developed a method capable of medium throughput screening for drugs targeting this stage, making our unit one of the few laboratories able to perform this research at a global level. To date, more than 10,000 molecules have been screened using our platform with the identification of promising hits. This activity is financed by MMV. In parallel, we have developed a research program—financed by the National Institute of Health (NIH)—with the University of Georgia to guide structure-activity relationships. This effort enables as well to better apprehend the biology of this elusive parasite stage (**Maher et al. eLife, 2024**)

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

Optimal drug regimen for *P. vivax* radical cure. PQ represents the gold-standard molecule used for the *P. vivax* radical cure. Beside the hepatotoxicity of this drug in G6PD-subjects, there is no consensus on the posology to adopt. Moreover, the individual drug efficacy is poorly characterized, notably because of the difficulty in proposing a suitable follow-up design. Understanding these aspects is critical for the elimination of *P. vivax*. To address these different points, we have developed a

collaborative project with the University of Maryland (UMD) which aims to determine the most effective PQ regimen. To avoid bias due to reinfection, we relocate enrolled patients in a no-transmission area during their entire follow-up. The first patients were recruited in November 2021, and 164 patients have been enrolled. The study is now completed and we have shown that high dose primaquine is needed in Cambodia leading to changes in the National Treatment Guidelines (**Eng et al, Lancet Infectious Disease, in press**).

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

Axis 2: Pre-clinical Vaccine Development for Plasmodium vivax

Blood-stage vaccine development. Thanks to the development of in vitro short-term cultures in the MRU in the past years, we are now able to evaluate blood-stage vaccine candidates to determine how they block parasite invasions in erythrocytes. We are currently focusing on PvDBP (**Martinez et al, npj Vaccines 2024; Martinez et al, Scientific Report, 2023Watson et al, Malaria J, 2023**), PvRBP2b (**Feufack et al, npj Vaccines 2024**), PvAMA1 (**Winnicki et al, Nature Communications 2024**) and PvRBP2a.

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

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

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

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

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

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

Axis 3: Host-Parasite Interactions

Factors involved in *P. vivax* growth within its host. We know very little about the factors influencing the growth of *P. vivax* in its human hosts. We started a project that aims at deciphering the contribution of human factors (specifically G6PD deficiency and HbE hemoglobinopathy) and of parasite genotypes on erythrocyte invasion and on the development of *P. vivax.* Using a combination of scRNA-seq, genotyping, and in vitro phenotype, we aim at better understanding how the parasite develops in its host. The first scRNA-seq libraries were sequenced in 2023 showing that the pipeline is fully functional, and the project will continue in the coming years.

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

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

The molecular mechanisms involved in the invasion of *P. vivax* into Duffy-negative erythrocytes are still unknown. Thanks to our ability to perform in-vitro short-term cultures of *P. vivax*, in collaboration with colleagues from Drexel University and Jimma University, we are aiming to identify the mechanisms that enable *P. vivax* to invade Duffy-negative red blood cells, an erythrocyte phenotype common in Africa, where increasing evidence of P. vivax infections is reported. Collection of parasites have been conducted in Ethiopia and samples have recently been shipped to IPC to perform in vitro analyses. Epidemiological analyses of parasites infecting Duffy negative and positive individuals are also a primary objective of this project (**Little et al, American j of Tropical Medicine and Hygiene, 2024**; **Pestana et al, Journal of Infectious Diseases, 2024; Tat et al, in preparation**) as well as understanding molecular mechanisms underlying P. vivax growth in these red blood cells (**Kepple et al, PLoS Neglected Tropical Diseases, 2024**).

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

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

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

Clinical protection against *P. vivax*. We and others have shown that some individuals can display clinical protection against *P. vivax* enabling them to be asymptomatic upon infection. We have started a project to determine the factors involved in this protection. Led by Jean Popovici and Tineke Cantaert at IPC, this project will study the genetic and immunologic determinants involved in this protection. Project is ongoing and results will be presented in future reports.

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

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

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

Axis 4: Operational Research for Malaria elimination (fueling basic biology research)

Efforts in the past years, including those conducted by IPC (Epidemiology Unit in collaboration with ours) have led to a massive reduction in P. falciparum malaria in the country (**Iv et al, The Lancet Regional Health-Western Pacific, 2024**). *P. vivax* is now the main species encountered in GMS. In Cambodia, it represents 90% of registered symptomatic cases. This parasite will be inherently more difficult to eradicate, not only because of its specific biology that causes chronic infections, but also because of the limitations in the methods developed so far. The overall aim of this axis is to fill this gap in order to help identify the most relevant future eradication strategies.

Deciphering malaria epidemiology in Cambodia. Our unit is part of the NIH Asia-Pacific ICEMR (Program Director: I. Mueller/ L. Robinson), which aims to address the key challenges to malaria elimination in the Asia-Pacific. We have applied serological investigations to evaluate the transmission of *P. vivax* in Eastern Cambodia and our results show that this tool provides great opportunity to understand fine-scale epidemiology (**Grimee et al, Malaria J, 2024**; **Tacoli et al, in preparation**). We have performed a final cross-sectional survey in 2023 to determine how much epidemiology has changed over time since the start of this study. In 2024, our ICEMR was renewed for an additional 5 years.

Name	ICEMR Asia-Pacific
Funding	NIH
Project duration	2017-2029
External collaborator	WEHI Australia (Ivo Mueller), Burnett Institute Australia (Leanne Robinson), IPP France (Michael White), CNM Cambodia (Dysoley Lek)

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

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

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

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

Surveillance of malaria infections among febrile illness in endemic areas. We have conducted in 2023 a surveillance study to determine the true burden of malaria infections among febrile treatment seeking individuals. The rationale of this work is that a significant number of infections might not be detected by current diagnostic tools (RDTs). We have enrolled nearly 1000 individuals from 6 Cambodian provinces and the main results are (i) current diagnostic tools detect only 40% of malaria cases and (ii) there seem to be an increasing number of non-falciparum non-vivax malaria cases compared to previous years (**Khim, Orban et al, in preparation**)

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

3.1.3 Research Programs – Outlooks for 2025

Are listed below projects that just started, however most projects indicated above are still ongoing.

Long-term culture of Plasmodium vivax. P. vivax cannot be cultured in vitro continuously. We recently obtained funding to pursue innovative efforts to establish long-term culture for this parasite (PI Jean Popovici, co-PI Jeremy Salvador).

Our approach relies on (i) access to a range of isolates from infected patients and (ii) mimicking in vitro bone marrow and splenic conditions that we believe are critical for parasite maintenance.

Name	PvCULT
Funding	NIH
Project duration	2025-2028
External collaborator	CNM Cambodia (Dysoley Lek)

Drug resistance in Papua New Guinea. Through a collaboration with Alyssa Barry (Deakin University) and Moses Laman (PNGIMR), we will investigate and characterize Plasmodium falciparum circulating in PNG in order to precisely determine antimalarial drug susceptibility and in case of resistance, the molecular determinants associated to it.

Name	PF_PNG
Funding	NHMRC
Project duration	2025-2028
External collaborator	Deakin (Alyssa Barry), PNGIMR (Moses Laman)

3.1.4 Support to National Authorities

Public Health Support for diagnostic of malaria. Through a contract with the WHO, we are the main laboratory in the Greater Mekong Sub-region (GMS) that provides support to national programs for characterizing drug resistance markers and phenotypes of Plasmodium falciparum. We are now expanding this support to also leverage our molecular diagnostic capacity to support national authorities in their diagnostics, especially of low-density parasite infections encountered in asymptomatic individuals screened during active surveillance surveys. We are endorsed as a WHO UKNEQAS referee lab to evaluate performance of diagnostic capacity in endemic settings as well as drug resistance molecular marker genotyping.

PvSeroTAT. Following our seminal study evaluating feasibility of PvSeroTAT in endemic area of Cambodia (**Tacoli et al, The Lancet Regional Health Western Pacific, in press**), we have been approached and endorsed by the WHO to provide serological testing on samples collected by malaria control programs in the GMS in order to treat potential P. vivax hypnozoite carriers and contribute to malaria elimination in the region. By being member of the VISPA consortium, with support from WEHI colleagues, we are now the first ones to perform these analyses in real-life programmatic settings.

3.1.5 Training and Teaching

PhD students:

SENG Dynang: University Paris-Saclay. Study of the biology of hypnozoites and relapses in Plasmodium vivax (Oct 2022-Oct 2025).

BALDOR Lea: University Paris-Saclay. Extent, dynamics and mechanisms of Plasmodium vivax immune evasion caused by PvDBP gene amplification (Oct 2023-Oct 2026)

Master & Engineer students:

	Name	Dates	Supervision level	Current position
Master 2	Suzanne Guepin	2024	50%	Pharmacy intern
	Sivkeng Ouk	2024	50%	Research Engineer
	Rominea Yeat	2024	50%	Research Engineer
	Baura Tat	2024	50%	Research Engineer
	Vanessa Gomes	2024	50%	unknown
	Rotha Eam	2024	50%	Ongoing
	Adelphe Anyang	2023-2024	50%	PhD student
	Lea Baldor	2023	50%	PhD student
Master 1	Sivkeng Ouk	2023	50%	Research Engineer
	Muhammad Nadeem	2023	50%	PhD student
	Rominea Yeat	2023	50%	Research Engineer
	Raphael Genin	2023	50%	unknown
	Justine Escard	2023	50%	PhD student

Teaching

Jean Popovici coordinated the TU Host-Pathogen Interactions of the Joint Master's offered by the UHS and the *Université de Paris-Saclay*. He provided 3 hours on antigenic variation in Plasmodium parasites and 3h on multipartite interactions for second-year master's students. He provided 3h lectures to first year Master students on Plasmodium parasites. He is jury member and one of the coordinators for IPC of this Master degree. **Brice Feufack, Dynang Seng, Nimol Khim and Agnes Orban**: supervised a 4*4h practical class on the techniques used to study malaria parasites

3.1.6 Outlook for upcoming 3 – 5 years

A number of ongoing projects listed above will continue for the next 3 to 5 years.

Our research will keep focusing on the challenges related to malaria in the GMS with a specific attention on *P. vivax*. These will keep combining basic laboratory research with applied, operational questions for elimination.

We are expanding our network of collaborators to leverage our knowledge and expertise (ie. in vitro culture of *P. vivax*) and use it at the service of other epidemiological contexts where malaria elimination is far from being achieved (ie. Ethiopia, Papua New Guinea).

In addition, we have to anticipate the complete elimination of *P. falciparum* in the region for the very near future and, though less likely, of *P. vivax*. In that regard, we expect to expand our research to other pathogens than strictly *Plasmodium* parasites.

Our immediate attention for this matter is to identify public health needs in Cambodia (and the region) with significant research gaps currently unaddressed at IPC to determine the most strategic decisions to be made for future research to be done in our unit. This could obviously include other parasitic infections, but also perhaps fungal infections or even bacterial pathogens.

3.1.7 Scientific Publications 2024

Note: The name of authors from the Institut Pasteur du Cambodge are underlined

1. **Indigenous emergence and spread of *kelch13* C469Y artemisinin-resistant *Plasmodium falciparum* in Uganda.**
   Awor P, Coppée R, Khim N, Rondepierre L, Roesch C, Khean C, Kul C, Eam R, Lorn T, Athieno P, Kimera J, Balikagala B, Odongo-Aginya EI, Anywar DA, Mita T, Clain J, Ringwald P, Signorell A, Lengeler C, Burri C, Ariey F, Hetzel MW, Witkowski B. Antimicrob Agents Chemother. 2024 Aug 7;68(8):e0165923. doi: 10.1128/aac.01659-23.
2. **The PvRBP2b-TfR1 interaction is not essential for reticulocytes invasion by Plasmodium vivax isolates from Cambodia.**
   Feufack-Donfack LB, Baldor L, Roesch C, Tat B, Orban A, Seng D, Salvador J, Khim N, Carias L, KingCL, Russell B, Nosten F, Ong AS, Mao H, Renia L, Lo E, Witkowski B, Popovici J.
   NPJ Vaccines. 2024 Nov 22;9(1):232. doi: 10.1038/s41541-024-01031-7.

3. **Potent AMA1-specific human monoclonal antibody against Plasmodium vivax Pre-erythrocytic and Blood Stages.**
   Winnicki AC, Dietrich MH, Yeoh LM, Carias LL, Roobsoong W, Drago CL, Malachin AN, Redinger KR, Feufack-Donfack LB, Baldor L, Jung NC, McLaine OS, Skomorovska-Prokvolit Y, Orban A, Opi DH, Kirtley P, Nielson K, Aleshnick M, Zanghi G, Rezakhani N, Vaughan AM, Wilder BK, Sattabongkot J, Tham WH, Popovici J, Beeson JG, Bosch J, King CL Nat Commun. 2024 Dec 4;15(1):10556. doi: 10.1038/s41467-024-53848-4.

4. **Plasmodium ovale spp dhfr mutations associated with reduced susceptibility to pyrimethamine in sub-Saharan Africa: a retrospective genetic epidemiology and functional study.**
   Joste V, Coppée R, Bailly J, Rakotoarivony Y, Toko Tchokoteu FG, Achache S, Pradines B, Cottrell G, Ariey F, Khim N, Popovici J, Mita T, Groger M, Ramharter M, Egbo T, Juma DW, Akala H, Houzé S, Clain J; Investigation Study Group.
   Lancet Microbe. 2024 May, https://doi.org/10.1016/s2666-5247(24)00054-5 . PMID: 38761813

5. ***Plasmodium vivax* spleen-dependent protein 1 and its role in extracellular vesicles-mediated intrasplenic infections.**
   Ayllon-Hermida A, Nicolau-Fernandez M, Larrinaga AM, Aparici-Herraiz I, Tintó-Font E, Llorà-Batlle O, Orban A, Yasnot MF, Graupera M, Esteller M, Popovici J, Cortés A, Del Portillo HA, Fernandez-Becerra C.
   Front Cell Infect Microbiol. 2024 May 17;14:1408451. https://doi.org/10.3389/fcimb.2024.1408451. eCollection 2024.PMID: 38828264

6. **Intermittent preventive treatment for forest goers by forest malaria workers: an observational study on a key intervention for malaria elimination in Cambodia**
   Sophea Iv, Chea Nguon, Phanith Kong, Téphanie Siena, Sreynet Srun, Céline Christiansen-Jucht, Chanvong Kul, Thornleaksmey Lorn, Sophy Chy, Jean Popovici, Amélie Vantaux, Benoit Witkowski, Antoine Berry, Patrice Piola, Claude Flamand.
   The Lancet Regional Health - Western Pacific Volume 47, June 2024, 101093 https://doi.org/10.1016/j.lanwpc.2024.101093

7. **Copy Number Variations of Plasmodium vivax DBP1, EBP/DBP2, and RBP2b in Duffy-positive and Duffy-negative Ethiopians.**
Pestana K, Ford A, Rama R, Abagero B, Kepple D, Tomida J, Popovici J, Yewhalaw D, Lo E.
J Infect Dis. 2024 Aug 5:jiae388. doi: 10.1093/infdis/jiae388. Online ahead of print.
PMID: 39102894

8. **Prevalence and Characteristics of Plasmodium vivax Gametocytes in Duffy-Positive and Duffy-Negative Populations across Ethiopia**
Ebony Little, Tassew T. Shenkutie, Meshesha Tsigie Negash, Beka R. Abagero, Abnet Abebe, Jean Popovici, Sindew Mekasha Feleke, and Eugenia Lo
AJTMH. 2024. 6;110(6):1091-1099. PMID: 38626749 PMCID: PMC11154031 DOI: 10.4269/ajtmh.23-0877

9. **Comparing malaria risk exposure in rural Cambodia population using GPS tracking and questionnaires.**
Pepey A, Souris M, Kim S, Obadia T, Chy S, Ea M, Ouk S, Remoue F, Sovannaroth S, Mueller I, Witkowski B, Vantaux A.
Malar J. 2024 Mar 12;23(1):75. doi: 10.1186/s12936-024-04890-6. PMID: 38475843

10. **Unraveling the intricacies of host-pathogen interaction through single-cell genomics.**
Gioacchino E, Vandelannoote K, Ruberto AA, Popovici J, Cantaert T.
Microbes Infect. 2024 Feb 16:105313. doi: 10.1016/j.micinf.2024.105313. Online ahead of print.
PMID: 38369008

11. **PvDBPII elicits multiple antibody-mediated mechanisms that reduce growth in a Plasmodium vivax challenge trial.**
Martinez FJ, White M, Guillotte-Blisnick M, Huon C, Boucharlat A, Agou F, England P, Popovici J, Hou MM, Silk SE, Barrett JR, Nielsen CM, Reimer JM, Mukherjee P, Chauhan VS, Minassian AM, Draper SJ, Chitnis CE.
NPJ Vaccines. 2024 Jan 6;9(1):10. doi: 10.1038/s41541-023-00796-7.

12. **Using serological diagnostics to characterize remaining high-incidence pockets of malaria in forest-fringe Cambodia.**
Grimée M, Tacoli C, Sandfort M, Obadia T, Taylor AR, Vantaux A, Robinson LJ, Lek D, Longley RJ, Mueller I, Popovici J, White MT, Witkowski B.
Malar J. 2024 Feb 15;23(1):49. doi: 10.1186/s12936-024-04859-5.

13. **In-vitro assessment of cutaneous immune responses to aedes mosquito salivary gland extract and dengue virus in Cambodian individuals**
David Guerrero, Sokchea Lay, Eakpor Piv, Chansophea Chhin, Sokkeang Leng, Ratana Meng, Kim Eng Mam, Polidy Pean, Amelie Vantaux, Sebastien Boyer, Dorothée Missé, Tineke Cantaert
Oxford Open Immunology, Volume 5, Issue 1, 2024, iqae003. Advance Access Publication Date: 1 April 2024 https://doi.org/10.1093/oxfimm/iqae003

14. **In Vitro Antimalarial Activity of Trichothecenes against Liver and Blood Stages of Plasmodium Species.**
    Parvatkar PT, Maher SP, Zhao Y, Cooper CA, de Castro ST, Péneau J, Vantaux A, Witkowski B, Kyle DE, Manetsch R.
    J Nat Prod. 2024 Feb 23;87(2):315-321. doi: 10.1021/acs.jnatprod.3c01019. Epub 2024 Jan 23. PMID: 38262446

15. **Comparative transcriptomics reveal differential gene expression among Plasmodium vivax geographical isolates and implications on erythrocyte invasion mechanisms.**
    Kepple D, Ford CT, Williams J, Abagero B, Li S, Popovici J, Yewhalaw D, Lo E.
    PLoS Negl Trop Dis. 2024 Jan 29;18(1):e0011926. doi: 10.1371/journal.pntd.0011926. Online ahead of print. PMID: 38285730

3.2 Epidemiology & Public Health Unit

3.2.1 Functional Structure

The EPHU conducts operational research on major public health challenges in Cambodia, focusing on cross-disciplinary epidemiological and clinical studies to inform interventions and policy recommendations. Our mission is to improve the understanding, prevention, diagnosis, and treatment of endemic and emerging infectious diseases in the general population, with a particular focus on vulnerable populations. Our research combines multiple disciplines, including infectious disease and environmental health epidemiology, clinical research, biostatistics, modeling, and social sciences, with a strong emphasis on the One Health approach. Recent studies have addressed key public health issues, such as rabies, arboviruses, HIV, tuberculosis, hepatitis, malaria, antimicrobial resistance, leptospirosis, influenza. In 2024, EPHU enhanced its strategy by initiating integrated and multisectoral research projects to improve surveillance, diagnostics, and data analysis, with a focus on advanced epidemiological methods to better understand disease transmission patterns and inform evidence-based public health interventions.

The year 2024 was also marked by the departure of Dr. Hélène Guis, veterinary epidemiologist, at the end of the first semester. Her team and the One Health group she led were successfully integrated into the Methodology and Data Analysis Group, enhancing the group's multidisciplinary efforts.

The unit, comprising 47 members in 2024, is structured into three complementary groups (Figure 5):

- **Community Epidemiology Group**, led by Dr. Ly Sowath, with expertise in community-based studies on rabies, dengue, avian influenza, and epidemic responses;
- **Clinical Research Group**, led by Dr. Nathalie de Rekeneire and Dr. Dim Bunnet, conducting clinical trials and guiding international strategies for the prevention, diagnosis, and treatment of diseases affecting vulnerable populations;
- **Methodology and Data Analysis Group**, coordinated by Dr. Claude Flamand, developing and implementing tailored methodological tools for study design, data management, and analysis.

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 viral hepatitis program, the National Animal Health and Production Research Institute (NAHPRI), the Council for the Development of Cambodia (CDC), the National Center for Parasitology, Entomology and Malaria Control (CNM), Malaria Consortium, national and reference hospitals in Phnom Penh and across the country, University of Health Sciences (UHS) and the Cambodia Academy of Digital Technology (CADT).

Conducted research projects result from several partnerships with international agencies or research groups including the Pasteur Network, the Wellcome Trust Fondation, the *Agence Nationale de Recherche sur le SIDA et les Maladies Infectieuses Émergentes* (ANRS-MIE), the International Vaccine Initiative (IVI), the European Union, *Institut National de la Santé et de la Recherche Médicale* (INSERM), CIRAD, IRD, the World Health Organization, *L'Initiative (Expertise France)*, the *Agence Française de Développement* (AFD), the French Embassy in Cambodia and the French Ministry of Foreign Affairs.

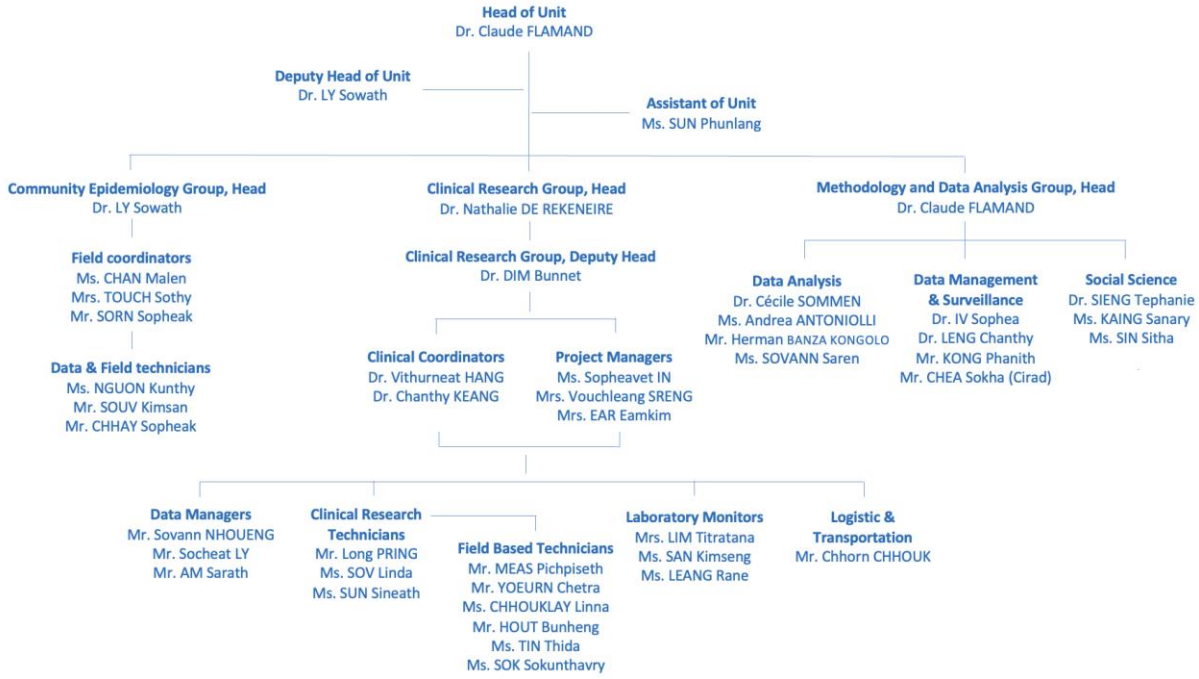


Figure 5: Epidemiology and Public Health Unit organigram, 2024

3.2.2 Research Programs - Major Achievements In 2024

Rabies

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

This study aims to evaluate the duration of the protective antibody response by following a cohort of approximately 170 patients at 14 days, 6 months, 1 year, and 3 years after receiving the first dose of the vaccine schedule. The cohort consists of patients who were bitten by dogs with a known rabies infection status and who received a 3-session intradermal rabies post-exposure prophylaxis (PEP) at IPC. As of February 2025, follow-up and blood sampling have been completed for years 1, 2, and 3 in 153, 110, and 124 participants, respectively. No PEP failures were encountered, and a protective level of immunity against rabies was observed among participants at one year (published).

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

KAP Rabies

A national survey was conducted in 2023-2024 to evaluate the burden of rabies exposure and human cases, the availability of rabies PEP, and healthcare workers' knowledge, attitudes, and practices (KAP). While 80% of health facilities reported treating patients with dog and cat bites, PEP was available in only 33.2% of these facilities. An estimated 151,235 individuals seek PEP annually, with 843 human rabies cases recorded in the past year. The survey identified significant knowledge gaps, with 70% unaware that asymptomatic rabid dogs can transmit the virus and 61% unaware that completing a full PEP course is 100% effective. An educational animated video, available in French,

Khmer, and English, was created and shared on major media platforms (https://www.pasteur-kh.org/rabies-prevention-centers/).

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

Arboviruses

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

We study dengue and dengue-like illness in Kampong Thom Province through a surveillance in children and adult inpatients at four referral hospitals, and through household investigations around dengue Index Cases found at hospitals. Sequential follow-up of positive cases were conducted for up to three years. During 2022-2024, 2,913 hospitalized patients were screened and 991 confirmed dengue infection by PCR. Community investigations around 119 Index Cases were conducted and screened 4,684 residents by PCR, of which 119 positive for dengue. DENV-2 was the most prevalent, followed by DENV-4. These results complement the National Dengue Control Program's data which relies primarily on syndromic passive surveillance in pediatric hospitals.

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

HIV and/or Tuberculosis

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

Mortality in people entering into HIV care late and who have a tuberculosis (TB) co-infection is high. The objective of the DATURA clinical trial is to estimate the impact of an intensified initial phase of tuberculosis (TB) treatment on mortality at 48 weeks among HIV-infected adults and hospitalized adolescents for TB with CD4 ≤ 100 cells/µL in comparison with the standard TB regimen. At the end of December 2024, among 209 patients who were pre-enrolled, 160 participants were included in this study. The recruitment of study participants was stopped on 17 December 2025 for futility following the DSMB recommendation. The follow-up of the last participant will end around December 2025.

Collaborations	Team leaders: Epidemiology and Public Health Unit: Nathalie de Rekeneire, Dim Bunnet (Clinical Research Group), Partners: NCHADS, Cambodia-China Friendship Preah Kossamak Hospital, Khmer-Soviet Friendship Hospital, Calmette Hospital, AID Health Care Foundation, NCHADS Clinic and Laboratory
Funding	ANRS-MIE (2021-2026)

Third TB National Prevalence Survey in Cambodia

The goal of the prevalence survey is to understand the burden and trends of tuberculosis (TB) and to develop a plan to improve TB control and care in line with the End TB Strategy targets. Dr. Bunnet DIM is the co-PI of this project. Our group is responsible for the data management, the mycobacteriology laboratory for cultures, and the IT aspects. The survey successfully completed 84 clusters in 35 weeks

(from June 11, 2023, to May 30, 2024). The preliminary results of the survey were presented at the Union World Conference on Lung Health 2024, held from November 12 to 16, 2024, in Bali, Indonesia. Dr. Bunnet also shared these preliminary results during the IPC scientific seminar on December 18, 2024.

Collaborations	Team leaders: Epidemiology and Public Health Unit: Nathalie de Rekeneire, Dim Bunnet (Clinical Research Group), Cheng Sokleaph (Deputy Head of Medical Biology Laboratory), Xavier Faure (Head of Informatics Technology) Partners: CENAT, RIT/JATA, HSD, CATA, USAID
Funding	DFAT (WHO), Global fund and USAID (2023-2024)

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

The aim of the project was 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 results of the study showed that an approach including introduction of 3HP, comprehensive health care workers training and PLHIV information based on previously identified barriers led to an increase in TPT coverage from 15% up to 86% in adult PLHIV attending HIV clinics. No proven efficacy of the introduction of the intervention itself within this step wedge cluster randomized trials (continued increase). A restitution meeting with all stakeholders and staff from all provinces was held in March 2024. This study has also enabled the restart of the HIV-TB technical working group which was on hold for quite a while.

Collaborations	Team leaders: Epidemiology and Public Health Unit: Dr. Laurence Borand, Dr. Dim Bunnet (Clinical Research Group) Partners: CENAT, NCHADS, CHAI
Funding	l'Initiative/Expertise France (2021-2024)

Hepatitis

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

Transmission from mother to child is the primary route of acquisition for both Hepatitis C (HCV) mono-infection and HCV/HIV co-infection in children. Cirrhosis occurs in less than 5% of children, but the proportion of patients with bridging fibrosis/cirrhosis has been reported to increase from 11% to 20% over a median period of 5.8 years. The objective of this study is to evaluate the effectiveness of the sofosbuvir/daclatasvir combination for children aged at least 6 years and for adolescents with active HCV infection. Recruitment started in August 2023. By the end of 2024, 17,553 children had been screened, and among them, 21 children were included in the therapeutic phase. We are working in close collaboration with the CDC National Viral Hepatitis Program, which refers children who test HCV positive at health centers to our study sites

Collaborations	Team Leaders: Nathalie de Rekeneire, Dim Bunnet (Clinical Research Group, IPC). Olivier SÉGÉRAL (University of Geneva) Partners: NCHADS and OI/ART sites, Jayavarman VII Hospital, Kantha Bopha 1 and 2 Hospitals, National Pediatric Hospital, Battambang Provincial hospital, CDC national viral hepatitis program
Funding	ANRS-MIE (2022-2026)

Fungal infections

FUNGICAM - ANRS 0465 - Prevalence of invasive fungal infection – Histoplasma spp., Talaromyces marneffei, Cryptococcus spp.- in severe immunocompromised HIV-infected patients in Cambodia

Histoplasmosis, talaromycosis and cryptococcosis are serious invasive fungal infections (IFIs) in patients with advanced HIV disease, but little available data on histoplasmosis and talaromycosis in Cambodia. Lack of awareness and insufficient availability of reliable diagnostic methods lead to delayed diagnosis. Antigenic and molecular methods emerged for the rapid diagnosis of IFIs. The objectives of this study are to: assess the prevalence of these IFIs in patients with advanced HIV disease in Cambodia, and raise awareness and train local actors on these infections and new and simple diagnosis tools. Biobank samples of the STATIS study, a study on the diagnostic management of tuberculosis in severely immunocompromised HIV-positive patients in Cambodia are analyzed and workshops for clinicians are planned in 2025.

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

Bacteriological Diseases and Antibiotic Resistance

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

This project assesses the socio-economic and regulatory aspects in relation to antimicrobial resistance (AMR) in low-and-middle-income countries (LMICs) and in One Health context. We conducted 75 interviews in three villages in Takeo province and had discussions with 27 Cambodian health professionals, including 7 lab personnels. As a result, communities self-medicate, guided by symptoms. Antibiotic usages by these communities underscored the significance of socio-economic and cultural factors influencing their understanding and actions that could lead to AMR. As example, farms raising market-bound chickens use antibiotics, while backyard raising for household consumption does not. Moreover, improper disposal of medicines by communities contributes to environmental contamination.

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

Malaria

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

Cambodia aims to eliminate *P. falciparum* by 2023 and all human malaria species by 2025, aligning with the WHO's Mekong Malaria Elimination program. This project focuses on identifying factors driving malaria transmission and assessing the effectiveness of Intermittent Preventive Treatment for Forest Goers (IPTf) in Cambodian forests. From March 2019 to January 2021, 2,198 Forest Goers participated in 3,579 interviews. The IPTf strategy led to a significant reduction in malaria prevalence, from 2.9% to 0.5% for P. falciparum and from 21.0% to 4.7% for P. ivax. Based on these results, the National Malaria Program has incorporated IPTf into its malaria elimination strategy. The data analysis was completed in 2024 and a publication presenting the findings was published in *Lancet Regional Health - Western Pacific*.

Collaborations	Epidemiology and Public Health Unit: P. Piola, S. Iv , C. Flamand, Malaria Unit (Benoit Witowski, Jean Popovici). Partners for Development (PfD), National Center for Parasitology, Entomology and Malaria Control (CNM), World Health Organization (WHO), Malaria Molecular Epidemiology Unit
Funding	*L'Initiative Canal 2*: 17SANIN205 / French Embassy – 2019-2024

Others

Info-ILLUS SROUL Project

The SROUL project, conducted in late 2024 with the Institut Pasteur Paris, aimed to explore local knowledge of infectious diseases and perceptions of scientific research. It involved two villages in Kampong Speu province: Koa Daun Tey, with no prior research experience, and Phsar Kontuot, where residents had participated in past studies. Focus groups were used to develop educational tools that improved understanding of topics like rabies, microbes, and vaccination. Participants in Koa Daun Tey showed strong curiosity and engagement, while those in Phsar Kontuot had a higher level of understanding due to previous research experience. The study demonstrated the effectiveness of a participatory approach in enhancing local knowledge and trust in public health initiatives. The tools and approach developed will be used in future research projects to improve community engagement and communication.

Collaborations	Epidemiology and Public Health Unit: Team leaders: T. Sieng , S. Ly, K. Nguon, C. Flamand, IP Paris (F. Momboisse), Inklink Association (L.Garancher)
Funding	*L'Initiative Canal 2*: 17SANIN205 / French Embassy – 2019-2024

Attitudes and Practices of Humans to Flying Foxes and Viral Threats in Battambang Province

A pilot study in 2024 investigates potential disease transmission from flying foxes to humans in Battambang province, Cambodia by using data on inhabitants' behaviors and perceptions about flying foxes and about viruses to create models predicting the probability of disease spillover. Forty residents in one village located next to a roost of flying foxes in Banan district were interviewed. Bayesian models showed that males were 90% more likely than females to have risk-taking behaviors. The next step is to incorporate bat pathogen data, validate the models and propose additional case studies.

Collaborations	Epidemiology and Public Health Unit: M. Chan, S. Ly Partners: Virology Unit (Duong V.), Provincial Health Department of Battambang, Nord University of Norway (Anneline Seppoia)
Funding	Nord University, Norway (2024)

3.2.3 Research Programs - Outlook for 2025

Hepatitis B

HBV universal vs Point-Of-Care-based Antiviral treatment to Prevent mother-to-child transmission: a multi-country cluster-randomized non-inferiority trial

To achieve global elimination of hepatitis B virus (HBV), it is crucial to eliminate HBV mother-to-child transmission (MTCT) by ensuring high coverage of birth dose vaccine and expanding the adoption of peripartum antiviral prophylaxis (PAP) by tenofovir. In their 2024 guidelines, the WHO issued a conditional recommendation for administering PAP to all HBsAg-positive women lacking access to viral load (VL) testing. As an innovative alternative, we propose the adoption of a rapid point-of-care test for hepatitis B correlated antigen (HBcrAg-RDT) to identify women eligible for PAP. The aim is to establish the non-inferiority of the HBcrAg-RDT strategy, in comparison to the universal strategy, in terms of effectiveness, defined as the reduction in maternal VL at the time of childbirth.

Collaborations	Team leaders: Nathalie de Rekeneire, Vithurneat Hang (Clinical Research Group, IPC) Partners: Dr. Yusuke Shimakawa (IP Paris), Dr. Didier Ekouevi (University of Lome, Togo), Dr. Olivier Ségéral (Geneva University), National Maternal and Child Health Center (NMCHC), National viral hepatitis program, National Immunization program (NIP)
Funding	ANRS-MIE (2025-2028)

One Health

Risk Assessment of Multiple Endemic Infectious Pathogens in a One Health Perspective (RACSMEI)

By 2025, the RACSMEI project aims to enhance Precision Public Health in Cambodia, a high-burden country for infectious diseases. By integrating human, animal, and environmental data through a One Health approach, the project will conduct a nationally-representative survey on several priority zoonotic and endemic diseases, covering 10,000 individuals, animals, vectors, and environmental factors. The research will utilize cutting-edge multiplex serology, environmental sampling, metagenomics and mathematical modeling to understand disease transmission dynamics, focusing on priority pathogens such as dengue, chikungunya, zika, japanese encephalitis, avian influenza, nipah virus, hantavirus, leptospirosis, and others. The findings will inform targeted interventions, strengthen surveillance, and contribute to informed public health policies and long-term collaborative research.

Collaborations	Team leaders: Epidemiology and Public Health Unit (S. Ly, T. Sieng, C. Flamand), CIRAD (H. Guis), Virology Unit (Erik Karlsson), LBM (S. Cheng, B. Guillard), Medical and Veterinary Entomology Unit (S. Boyer), Malaria Consortium, MoH, MaFF, MoE
Funding	Wellcome TrustDiscovery Award, AFD-Ecomore : 2024-2030

AFRICAM-PREACTS- PREZODE

AFRICAM is a One Health project developed under the framework of the Preventing Zoonotic Disease Emergence (PREZODE) initiative, coordinated by CIRAD and IRD in four African countries and Cambodia. The main objectives of the epidemiological component of the project aims to study zoonotic risks at various interfaces between humans, animals, and the environment using a 2-year longitudinal serological follow-up including 450 households from 3 villages of Battambang. The protocol was submitted to the ethics committee in 2024 and the serological longitudinal follow-up phase will start in the first semester in Battambang province.

Collaborations	Epidemiological Survey - Team Leaders: S. Ly, A. Antoniolli, K. Nguon, C. Flamand; PREZODE - AFRICAM - Team leaders : Anne-Laure Banuls (IRD), Flavie Goutard (Cirad), H. Guis and V. Herbreteau. Partners in Cambodia: IRD, CIRAD, IPC, *Agronomes et Vétérinaires sans Frontières* (AVSF), International Development Enterprises (IDE), ITC, Wildlife Conservation Society (WCS), Battambang Hospital.
Funding	AFD, Foundation Simone Del Duca : 2025-2028

VIRAGE

The VIRAGE project aims to address critical gaps in rabies awareness, post-exposure prophylaxis (PEP) access, and risk management in Cambodia as part of the "Zero by 30" initiative. Building on the findings of the Cap-Rage project, VIRAGE focuses on investigating public knowledge, attitudes, and practices through a nationwide survey, enhancing surveillance, and evaluating the effectiveness of rabies control interventions. The project seeks to expand PEP access, train health professionals, and raise awareness, particularly in rural areas. By adopting a One Health approach, VIRAGE integrates human and animal health perspectives and explores socio-behavioral factors affecting PEP access. It aims to strengthen intersectoral collaboration and provide data for improving rabies control strategies.

Collaborations	Team Leaders : C. Flamand, S. Ly, T.Sieng, H. Guis (CIRAD). In coll. with CDC MoH and GDAHP MAFF
Funding	French Ministry of Europe and Foreign Affairs through the Solidarity Funds for Innovative Projects (FEF) :2025-2027

3.2.4 Support to National Authorities

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

- N. de Rekeneire and Dim B. are members of the Cambodian Committee for TB Research (CCBR). They are also part of the technical working group (TWG) on HIV.
- Dim B. is involved with the CENAT for the TB prevalence survey that started in May 2023.
- N. de Rekeneire and Dim B. participate to the writing of the next hepatitis elimination guidelines, coordinated by the national viral hepatitis program, CDC MoH
- Ly S. worked with the Emergency Operations Center (EOC) for Covid-19, coordinated by the Cambodian CDC-MoH.
- Ly S. and V. Herbreteau teams provided support for data management for Covid-19 lab testing at IPC and ensured the transfer of quality data to the CDC-MoH.

- C. Flamand participated in Dengue and Influenza surveillance workshops organized by CDC-MoH and WHO and the consultative committee on One Health Strategy for Cambodia organized by MoH.
- C. Flamand and S. Ly contributed to the consultation for the Technical Working Group on the Human Rabies Vaccine by the MoH.
- S. Ly and C. Flamand contributed to the consultation from the MoH for the dengue vaccine strategy.
- T. Sieng participated in the AMR Technical Working Group coordinated by MoH.

3.2.5 Teaching and Training

Teaching and Training

- Ly Sowath, Lectures for foundation year students at the University of Health Science,
- Dim Bunnet, Teaching Clinical Research at the Master of Infectiology, University of Health Science & Paris-Saclay University
- Sovann Nheuong, Teaching Data management in clinical research at the Master of Infectiology, University of Health Science & Paris-Saclay University
- Socheat Ly, Teaching Data management in clinical research at the Master of Infectiology, University of Health Science & Paris-Saclay University
- Claude Flamand, Teaching in Biostatistics & Epidemiology at the Master I & II of Infectiology, University of Health Science & Paris-Saclay University
- Nathalie de Rekeneire, Teaching in Clinical Trials Methodology at the Master of Infectiology, University of Health Science & Paris-Saclay University
- Nathalie de Rekeneire, Teaching at the Master 2 Santé Globale dans les Suds- Santé mondiale of ISPED
- Cecile Sommen, Andrea Antoniolli & Claude Flamand, Training in Biostatistics with R, Institut Pasteur in Cambodia, November 2024

PhD students

- Mrs Iv Sophea, Paul Sabatier-Toulouse III University, 2019-2024 – 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), Defended November, 18, 2024
- Ms Andréa Antoniolli; Paris Cité University, ED 393, 2022 – * Thesis title: Transmission of leptospirosis in Cambodia. (Director: C. Flamand)
- Mr Herman Banza Kongolo, Paris Cité University, ED 393, 2022 – * Thesis title: Estimation of the rabies load burden in Cambodia (Director: C. Flamand, Co-supervisor: H. Guis)
- Mr Sopheak SORN, Paris Cité University, ED 393, 2023 – * Thesis title: Transmission dynamics and determinants of arboviruses in Cambodia (Director: C. Flamand, Co-supervisor: Dr Sowath Ly)
- Ms Malen CHAN, Paris Cité University, ED 393, 2024 - * Thesis title: Transmission dynamics and determinants of arboviruses in Cambodia (Director: C. Flamand, Co-supervisor: Dr Sowath Ly)

Master's students

- Mr Nheuong Sovann (UHS-Epidemiology, 2020–2025). Master thesis defended in 2025
- Ms Sov Linda (Master of Science in Epidemiology at National Institute of Public Health, 2020–2023). Master thesis defense in February 2024.

- Ms In Sopheavet (Master in Epidemiology, National Institute of Public Health, 2022-2024)
- Ms Sun Sineath (Master of Health and Community Development, National Institute of Public Health, 2022–2024)
- Mr Long Pring (Master in Epidemiology, National Institute of Public Health, 2022–2024)
- Ms San Kimsey (Master in Public Health, National Institute of Public Health, 2022–2024)
- Mr Lay Virak (Master in Epidemiology, National Institute of Public Health, 2022–2024)
- Mrs. Sreng Vouchleang (Master in Epidemiology, National Institute of Public Health, 2023–2025)
- Ms Chan Malen (Master of Applied Epidemiology - MAE, ASEAN–Australia Health Security Fellowship Program, National University of Australia - ANU, 2022–2024)
- Ms Hoen Thavry (Master II in Epidemiology, National Institute of Public Health, 2023–2024)
- Ms Josephine JUËT, Pharmaceutical Sciences, Industry Track, Paul Sabatier University Toulouse, 2023-2024

3.2.6 Outlook for upcoming 3 – 5 years

The research strategy of the EPHU for the upcoming years will focus, in the short term, on implementing integrated, multidisciplinary research programs aligned with Cambodia's identified priorities. The launch of key projects such as RACSMEI, VIRAGE, AFRICAM and HIPOCAMP in 2025 presents a significant challenge for the development of the unit's research strategy and the growth of the team's capabilities. These programs aim to deepen our understanding of priority infectious diseases and inform targeted strategies for disease surveillance, prevention, and management. Addressing the significant challenges of upcoming projects will require strengthening the team's capacity, particularly in statistical data analysis and environmental sciences, by fostering the training of young talents within the methodology and data analysis group. Recruiting a senior statistician and a geohealth specialist will be crucial to this strategy, particularly in addressing future challenges, such as studying the interactions between health, the environment, and climate. Hosting international post-doctoral researchers will also be essential for further strengthening the capacity-building strategy.

In the medium term, the promotion of collaborative, multi-scale One Health projects will position the unit at the forefront of field-based One Health approaches, an area still underdeveloped. These projects will strengthen the unit's research strategy and enhance its regional positioning, reputation, and impact. Integrating social sciences and community engagement will also be a key priority. A "Social sciences" group will be created within the unit, specializing in combining qualitative and quantitative approaches. In clinical research, it will be essential to initiate and coordinate in-house projects to enhance the scientific output of the clinical research group and foster capacity-building. While already recognized by ANRS as a leading study-site for multicentric studies, the focus will be on expanding its leadership and research capabilities through new initiatives.

Beyond three years, the results from these approaches should allow for the **evaluation of the impact of future intervention strategies** and **strengthen collaborations with health authorities** in identifying the **best precision and culturally-appropriate public health strategies** aimed at reducing transmission risks in the most exposed populations. Finally, in the longer term, given the critical public health issues in Cambodia, it is imperative to explore opportunities to broaden research themes to include non-communicable diseases (respiratory diseases, cardiovascular diseases, cancers, etc.).

3.2.7 Scientific Publications 2024

Note: *The name of authors from the Institut Pasteur du Cambodge are underlined*

1. **Perceptions, facilitators and barriers to the implementation of interpersonal group therapy to treat depression among people living with HIV in Senegal: a qualitative study.**
   Bernard C, Mané I, Ziadeh S, Tine JM, Diaw A, Benzekri N, Ndiaye I, Samba O, Font H, Bottai T, Jacquesy L, Verdeli H, Ngom NF, Dabis F, Seydi M, de Rekeneire N. Front Public Health. 2024 Jan 24;12:1295181. doi: 10.3389/fpubh.2024.1295181.

2. **Simplified Criteria to Assess Long-Term Antiviral Treatment Indication in Chronic HBV-Infected Pregnant Women in Cambodia.**
   J.S. Yang, S. Sovann, Y. Shimakawa, S. Nhoueng, B. Dim, C.Vong, C. Sann, J. Guillebaud, D. Vann, B. Touch, H. Chea, W. Pisey Choupoan Phirum, E. Rosenthal , C. Paul, L. Khun, C. Yay, D. Laurent, S. Chhun, L. Borand and O. Segeral** Viruses 2024, *16*(2), 194; https://www.mdpi.com/1999-4915/16/2/194

3. **Evaluation of a short training course of chest X-ray interpretation for the diagnosis of paediatric TB.**
   Melingui, B. F; Leroy-Terquem, E.; Palmer, M.; Taguebue, J-V.; Wachinou, A. P.; Gaudelus, J.; Salomao, A.; Bunnet, D.; Eap, T. C.; Borand, L.; Chabala, C.; Khosa, C.; Moh, R.; Mwanga-Amumpere, J.; Eang, M. T.; Manhiça, I.; Mustapha, A.; Beneteau, S.; Falzon, L.; Seddon, J. A.; Berteloot, L.; Wobudeya, E.; Marcy, O.; Bonnet, M.; Norval, P. Y. IJTLD OPEN, Volume 1, Number 2, 1 February 2024, pp. 76-82(7) https://doi.org/10.5588/ijtldopen.23.0484.

4. **Challenges of rabies surveillance in Madagascar based on a mixed method survey amongst veterinary health officers.**
   Dreyfus A, Volasoa MH, Guis H, Razafindraibe NP, Razafindramparany MH, Arivony NL, Rakotondrabe N, Andriamananjara MA, Dussart P, Kassie D, Lacoste V, Andriamandimby SF. Front Vet Sci. 2024 Feb 28;11:1270547. doi: 10.3389/fvets.2024.1270547. eCollection 2024. PMID: 38487708.

5. **Use of Herbal Medicine in French Guiana: Influences and Challenges for Prevention Strategies in the Context of the COVID-19 Pandemic.**
   Forsans, M.-A. Tareau, L. Ramiz, C. Alves Sarmento, N. Clément, A. Perilhou, N. Vignier, G. Odonne, M. Nacher, C. Flamand. Journal of Herbal Medicine Volume 44, March 2024, 100848 DOI: 10.1016/j.hermed.2024.100848.

6. **Effect of decentralising childhood tuberculosis diagnosis to primary health centre versus district hospital levels on disease detection in children from six high tuberculosis incidence countries: an operational research, pre-post intervention study.**
   Wobudeya E, Nanfuka M, Ton Nu Nguyet MH, Taguebue JV, Moh R, Breton G, Khosa C, Borand L, Mwanga-Amumpaire J, Mustapha A, Nolna SK, Komena E, Mugisha JR, Natukunda N, Dim B, de Lauzanne A, Cumbe S, Balestre E, Poublan J, Lounnas M, Ngu E, Joshi B, Norval PY, Terquiem EL, Turyahabwe S, Foray L, Sidibé S, Albert KK, Manhiça I, Sekadde M, Detjen A, Verkuijl S, Mao TE, Orne-Gliemann J, Bonnet M, Marcy O; TB-Speed Decentralisation study group. EClinicalMedicine.

2024 Mar 21;70:102527. doi: 10.1016/j.eclinm.2024.102527. eCollection 2024 Apr. PMID: 38685921.

7. **Cost-effectiveness and budget impact of decentralising childhood tuberculosis diagnosis in six high tuberculosis incidence countries: a mathematical modelling study.**
d'Elbée M, Harker M, Mafirakureva N, Nanfuka M, Huyen Ton Nu Nguyet M, Taguebue JV, Moh R, Khosa C, Mustapha A, Mwanga-Amumpere J, Borand L, Nolna SK, Komena E, Cumbe S, Mugisha J, Natukunda N, Mao TE, Wittwer J, Bénard A, Bernard T, Sohn H, Bonnet M, Wobudeya E, Marcy O, Dodd PJ; TB-Speed Health Economics Study Group. EClinicalMedicine. 2024 Mar 21;70:102528. doi: 10.1016/j.eclinm.2024.102528. eCollection 2024 Apr. PMID: 38685930.

8. **Mixed methods to evaluate knowledge, attitudes and practices (KAP) towards rabies in central and remote communities of Moramanga district, Madagascar.**
Leblanc C, Kassié D, Ranaivoharimina M, Rakotomanana EFN, Mangahasimbola RT, Randrianarijaona A, Ramiandrasoa R, Nely AJ, Razafindraibe NP, Andriamandimby SF, Ranoaritiana DB, Rajaonarivony V, Randrianasolo L, Baril L, Mattern C, Ratovoson R, Guis H. PLoS Negl Trop Dis. 2024 Mar 29;18(3):e0012064. doi: 10.1371/journal.pntd.0012064. Online ahead of print. PMID: 38551968

9. **Bayesian modeling of post-vaccination serological data suggests that yearly vaccination of dog aged <2 years old is efficient to stop rabies circulation in Cambodia.**
Auerswald H, Guillebaud J, Durand B, Le Vu M, Sorn S, In S, Pov V, Davun H, Duong V, Ly S, Dussart P, Chevalier V. PLoS Negl Trop Dis. 2024 Apr 18;18(4):e0012089. doi: 10.1371/journal.pntd.0012089. eCollection 2024 Apr. PMID: 38635851.

10. **Climate-driven models of leptospirosis dynamics in tropical islands from three oceanic basins.**
Douchet L, Menkes C, Herbreteau V, Larrieu J, Bador M, Goarant C, Mangeas M. PLoS Negl Trop Dis. 2024 Apr 25;18(4):e0011717. doi: 10.1371/journal.pntd.0011717. Online ahead of print. PMID: 38662800.

11. **Rapid decrease in IL-1Ra and IP-10 plasma levels following tuberculosis treatment initiation.**
P. Polidy, R. Affi, C. Chazalon, B. C. Soumahoro, D. Gabillard, D. Bunnet, L.Borand, R. Moh, X.Anglaret, F.X Blanc, P.M Girard, G. Carcelain, D. Laureillard, L. Weiss
International Journal of Infectious Diseases Available online 11 May 2024, 107096 https://doi.org/10.1016/j.ijid.2024.107096

12. **Increased frequencies of highly activated regulatory T cells skewed to a T helper 1-like phenotype with reduced suppressive capacity in dengue patients.**
Sann S, Heng B, Vo HTM, Arroyo Hornero R, Lay S, Sorn S, Ken S, Ou TP, Laurent D, Yay C, Ly S, Dussart P, Duong V, Sakuntabhai A, Kleinewietfeld M, Cantaert T. mBio. 2024 May https://doi.org/10.1128/mbio.00063-24 . PMID: 38752787

13. **Intermittent preventive treatment for forest goers by forest malaria workers: an observational study on a key intervention for malaria elimination in Cambodia.**
S. Iv, C.Nguon, P. Kong, T. Sieng, S. Srun, C. Christiansen-Jucht, C. Kul, T. Lorn, Sophy Chy,

J. Popovici, A. Vantaux, B. Witkows, A. Berry, P.Piola, C. Flamand.
The Lancet Regional Health - Western Pacific Volume 47, June 2024, 101093 https://doi.org/10.1016/j.lanwpc.2024.101093

14. **Spatiotemporal Modeling of Aedes aegypti Risk: Enhancing Dengue Virus Control through Meteorological and Remote Sensing Data in French Guiana.**
Bailly S, Machault V, Beneteau S, Palany P, Fritzell C, Girod R, Lacaux JP, Quénel P, Flamand C. Pathogens 2024, 13, 738, https://doi.org/10.3390/pathogens13090738

15. **Mosquito dynamics and their drivers in peri-urban Antananarivo, Madagascar: insights from a longitudinal multi-host single-site survey.**
Tantely ML, Guis H, Raharinirina MR, Ambinintsoa MF, Randriananjantenaina I, Velonirina HJ, Revillion C, Herbreteau V, Tran A, Girod R. Parasit Vectors. 2024 Sep 10;17(1):383. doi: 10.1186/s13071-024-06393-4.

16. **TB-Speed Decentralization Study Group. Implementation of digital chest radiography for childhood tuberculosis diagnosis at district hospital level in six high tuberculosis burden and resources limited countries.**
Melingui BF, Basant J, Taguebue JV, Massom DM, Leroy Terquem E, Norval PY, Salomao A, Dim B, Tek CE, Borand L, Khosa C, Moh R, Mwanga-Amumpere J, Eang MT, Manhiça I, Mustapha A, Balestre E, Beneteau S, Wobudeya E, Marcy O, Orne-Gliemann J, Bonnet M. Trop Med Int Health. 2024 Nov;29 (11):979-989. doi: 10.1111/tmi.14053.

17. **Spatiotemporal evolution and transmission dynamics of Alpha and Delta SARS-CoV-2 variants contributing to sequential outbreaks in Cambodia during 2021.**
Su YCF, Zeller MA, Ou TP, Ma J, Pum L, Zhang R, Rath S, Heang V, Kol S, Lim R, Chea KL, Khun L, Heng L, Krang S, Raftery P, Kinzer MH, Ieng V, Kab V, Patel S, Sar B, Horm VS, Yann S, Auerswald H, Siegers JY, Troupin C, Boukli N, Vandelannoote K, Wong FY, Ng GGK, Chan M, Sorn S, Sengdoeurn Y, Heng S, Darapheak C, Savuth C, Khalakdina A, Ly S, Baril L, Spiegel A, Duong V, Ly S, Smith GJD, Karlsson EA. Commun Med (Lond). 2024 Nov 28;4(1):252. doi: 10.1038/s43856-024-00685-7.

18. **Evaluation of one year immunity following rabies post-exposure prophylaxis in dog bite cases.**
Ya N, Auerswald H, Touch S, In S, Yun C, Thai P, Sann S, Heng B, Leng C, Duong V, Peng YS, Ly S, Cantaert T. NPJ Vaccines. 2024 Nov 27;9(1):237. doi: 10.1038/s41541-024-01030-8.

19. **One Health Field Approach Applied to Leptospirosis: A Systematic Review and Meta-Analysis Across Humans, Animals and the Environment.**
Antoniolli A, Guis H, Picardeau M, Goarant C, Flamand C. Open Forum Infect Dis. 2024. 12(1). 2328-8957. doi: 10.1093/ofid/ofae757

3.2.8 Oral Communications and Posters

1. **Descriptive analysis of rabies post-exposition prophylaxis in Cambodia from 1998 to 2022.**
Banza Kongolo H, Guis H, Chan M, Thai P, Thap V, Yin H, Girond F, Ly S, Peng Y, Flamand C. Poster, Paris-Cité University, ED393 PhD School Seminar, Saint Malo, France 5-7 février 2024.

2. **One Health characterization of leptospirosis transmission in humans, animals and the environment: a systematic review with meta-analysis.**
   Antoniolli A, Guis H, Goarant C, Flamand C. Poster, Paris-Cité University, ED393 PhD School Seminar, France, 5-7 février 2024

3. **Uptake of cervical cancer screening services by female sex workers in the two main cities in Burkina Faso: a cross-sectional study.**
   Traore IT, Aida M. Traore AM, Wilfried Wenceslas Bazié WW, Ajani Ousmane Taofiki O, Ivette Zoundi I, de Rekeneire N, Vuylsteke B, Alary M, Nicolas Nagot N. Mini-oral communication for poster, INTEREST conference, Cotonou, Benin, 14-17 May, 2024

4. **Epidemiological Findings of the DENTHOM Cohort Study.**
   Sorn S, Nguon K, Chhay S, Ken S, Lay S, Duong V, Cantaert T, Flamand C, Ly S. Poster Presentation, CREID Network 2024 Annual Meeting, USA, June 10-12, 2024.

5. **Within-household transmission of chikungunya virus during an outbreak in Cambodia.**
   Sorn S, Nguon K, Chhay S, Ken S, Ou T, Duong V, Watson H, Ly S, Flamand S. Oral Presentation and Competition, ICTMM2024, Kuching, Malaysia, September 19-23, 2024.

6. **Estimating rabies post-exposure prophylaxis delivery capacity: A National survey among health staff in Cambodia.**
   Kaing S, Leng C, Kong P, Yi S, Banza Kongolo H, Chan M, Peng Y, Ly S, Guis H, Flamand C. Oral Presentation, ICTMM2024, Kuching, Malaysia, September 19th-23rd, 2024.

7. **Knowledge, Attitudes and Practices towards rabies among healthcare workers in Cambodia – a national survey.**
   Kong P, Leng C, Kaing S, Yi S, Peng Y, Chan M, Banza Kongolo H, Ly S, Flamand C, Guis H. Poster Presentation and Competition, ICTMM2024, Kuching, Malaysia, September 19th-23rd, 2024.

8. **One Health field approach applied to Leptospirosis: a systematic-review and meta-analysis across humans, animals and the environment.**
   Antoniolli A, Guis H, Picardeau M, Goarant C, Flamand C. Oral presentation, ICTMM2024, Kuching, Borneo, Malaysia, September, 19-23th, 2024.

9. **25 years of rabies post-exposure prophylaxis in Cambodia: 1998 - 2022.**
   Banza Kongolo H, Flamand C, Peng Y, Chan M, Heidi A, Veasna D, Thai P, Thap V, Yin H, Ly S, Guis H. Oral presentation, ICTMM 2024, Kuching, Malaysia, September, 19-23rd, 2024.

10. **One Health field approach applied to Leptospirosis: a systematic-review and meta-analysis across humans, animals and the environment.**
    Antoniolli A, Guis H, Picardeau M, Goarant C, Flamand C. Rapid oral presentation and poster, ISVEE17, Sydney, Australia, November 11-15th, 2024.

11. **Healthcare seeking behavior and gender differences among people with presumptive TB: Preliminary results from the 3 rd national TB prevalence survey in Cambodia.**
Khun KE, An Y, Onn S, Pheng S , Norio Y, Aung KM, Narith R, Deng S, Huot CY, Dim B, Onozaki I. E-poster, 55th Union World Conference on Lung Health, 12-16 November 2024

12. **Gender differences of TB risk factors: Preliminary results from 52 clusters of the 3rd national TB prevalence survey in Cambodia.**
An Y, Eam KE,Onn S,. Pheng S, Yamada N,Aung KM, Narith R, Deng S, Yuda CY, Dim B, Onozaki I. E-poster, 55th Union World Conference on Lung Health, 12-16 November 2024

13. **Characteristics of asymptomatic TB cases from the third national TB prevalence survey and their implications to active case detection in Cambodia.**
Aung KM, Siphann O, Rithy O, Satha P, Onozaki I, Yamada N, Dim B, Yom A, Khun KE, Huot CY. Oral presentation, 55th Union World Conference on Lung Health, 12-16 November 2024

14. **The Results of the 3rd TB National Prevalence Survey of Cambodia 2023.**
Huot C, Khun KE, Ikushi O, Dim B. 2024 at the Union World Conference on Lung Health, BALI November 12 - 16, 2024. SP28-587-16

15. **An overview of TA-PHROM and its subsequent developments in HIPOCAMP.**
Dim B, Ségéral O. ANRS MIE Scientific Days. Siem Reap, Cambodia. October 10-11, 2024

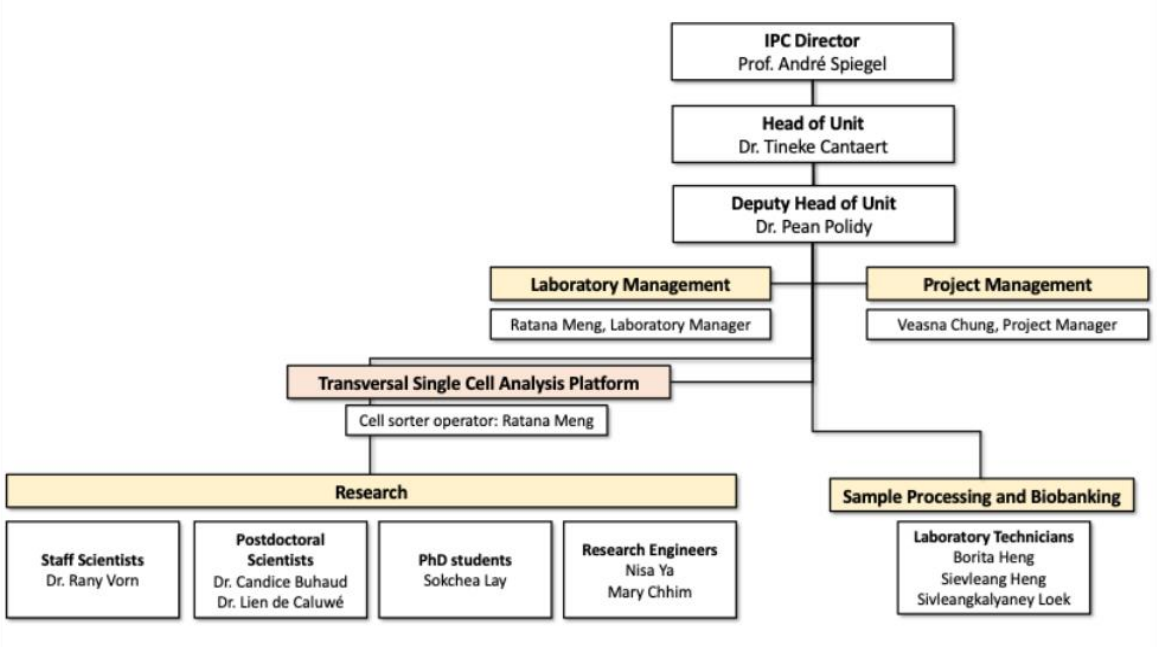
16. **Opticam study.**
Sreng V. ANRS MIE Scientific Days. Siem Reap, Cambodia. October 10-11, 2024

17. **Determination of Adequate Tuberculosis Regimen in Adults (DATURA).**
In S**.** ANRS MIE Scientific Days. Siem Reap, Cambodia. October 10-11, 2024.

18. **Comparative Anthropology in Southeast Asia.**
Sieng T. Center for Southeast Asian Studies (UMR 8170 EHESS/CNRS)- École française d'Extrême-Orient (EFEO), Paris, France. May 16, 2024.

3.3 Immunology Unit

3.3.1 Functional Structure



3.3.2 Research Programs – Major Achievements in 2024

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

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

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

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

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

Moreover, increased IgG1 afucosylation levels correlated with increased hematocrit and decreased platelet counts, 2 hallmarks of dengue disease severity. (Bournazos et al, Science 2021). In the continued collaboration with Rockefeller University, USA, we now assessed the in vivo mechanisms of antibody-mediated dengue pathogenesis. we discovered that the pathogenic activity of anti-DENV antibodies is exclusively mediated through engagement of FcγRIIIa on splenic macrophages, resulting in inflammatory sequelae and mortality. These findings highlight the importance of IgG–FcγRIIIa interactions in dengue, with important implications for the design of safer vaccination approaches and effective therapeutic strategies (Yamin et al, Nature Microbiol 2023).

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

Evaluation of regulatory T cells in dengue

Regulatory T cells (Tregs) are essential in maintaining immune homeostasis and peripheral tolerance. In the context of infectious diseases, Tregs have both beneficial and harmful effects: while they dampen excessive immune activation, they also can suppress beneficial antigen-specific immune responses. Given the current increased understanding of different Treg subsets and their functional properties, we aimed to define in detail the phenotype and function of different Treg subsets and their association with disease severity in a unique cohort of more than forty Cambodian children undergoing an acute secondary dengue infection with variable disease outcome. Given the complexity of dengue immunopathology and the development of exacerbated immune responses in severe dengue, our published data clarify the role of regulatory T cells in dengue immunopathogenesis (Sann et al, mBio, 2024).

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

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

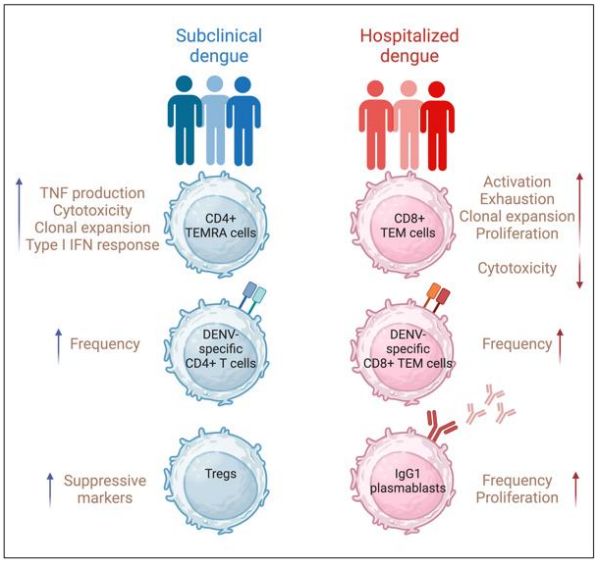


Figure 6: Single cell analysis of subclinical and hospitalized dengue cases reveals altered frequencies and functions of T and B cell subsets in severe dengue (Gonnella et al, submitted).

IP Cambodia is part of the NIH funded consortium PI-CREID. Here, the Immunology Unit is involved in the study of host adaptive immune responses to emerging infectious diseases in South-East Asia. In this framework, we have established a single cell analysis and monoclonal antibody production platform at IP Cambodia. We will increase our insight into the adaptive immune response (both B and CD4 T cell responses) at a single cell level and the sequence-function relationship of human antibodies generated during arbovirus infections by combining sequencing at a single cell level with antibody repertoire analysis.

Investigation of adaptive immune signatures associated to protection after DENV infection

To define adaptive immune signatures associated to protection from hospitalized dengue, we performed in depth immunoprofiling, single cell T cell receptor (TCR) and B cell receptor (BCR) analysis and quantified DENV-specific T cells in Cambodian children with subclinical infection or hospitalized dengue. We uncover that individuals with subclinical infection exhibit increased frequency and clonal expansions of CD4+ TEMRA cells and DENV-specific CD4+ T cells, and

demonstrate a gene expression signature showing increased Treg functionality. Across all T cell subsets, subclinical cases upregulated a type I IFN response gene signature. In contrast, expanding CD8+ EM cells from hospitalized patients show signs of exhaustion and are functionally impaired. In addition, hospitalized dengue is characterized by high frequencies and clonally expanded IgG1-expressing plasmablasts. These findings identify novel candidate correlates of protection and support a rationale for T cell directed interventions for dengue disease (Gonnella et al, submitted).

Start/End Year	2020-2025
Collaborations	Virology Unit, IP Cambodia (DUONG V), Epidemiology and Public Health Unit, IP Cambodia (LY S), Entomology Unit, IP Cambodia (BOYER S), Institut Pasteur Paris (SAKUNTABHAI A, SIMON-LORIERE E, HASAN M)
Funding	NIH PICREID (1U01AI151758 – 01): 2020-2025

1.2. *Immunity to Aedes mosquito saliva*

When a mosquito inserts its proboscis and probes for blood, the mosquito ejects a salivary mix of vasodilators, anticoagulants, immunomodulatory and anti-hemostatic components into both the epidermis and the dermis (reviewed in Cantaert T and Manning J, Vaccines, 2019). However, little is known about skin immunity to mosquito saliva. In 2022, we assessed the cutaneous innate and adaptive immune responses via controlled *Aedes aegypti* feedings in humans living in an Aedes-endemic country (Guerrero, Vo et al, Nat Comm 2022). In a follow up study of this work, our objective was to gain a deeper insight into the impact of different strains of *Aedes* mosquitoes and DENV on the local human skin immune response, particularly in individuals residing in regions where *Aedes* mosquitoes and dengue are prevalent. Our findings revealed that exposure to *Aedes* mosquito saliva led to significant changes in the characteristics of immune skin cells. These changes included a notable increase in the number of monocytes expressing the CCR2 marker, a higher proportion of neutrophils expressing CD69, and a substantial increase in the proportion of infected monocytes isolated from the human skin biopsies (Guerrero et al, Oxf Open Immunol, 2024)

Start/End Year	2020-2024
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

1.3. Pasteur International Unit

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

Start/End Year	2023-2029
Collaborations	Immunopathophysiology and infectious diseases dpt, Institut Pasteur de Dakar (VIGAN-WOMAS I)

	Immunology and Infectious Disease Unit, Institut Pasteur de Madagascar (SCHOENHALS M)
Funding	Institut Pasteur du Cambodge/Pasteur Network

Axis 2: Biomarkers of Infectious Diseases

2.1. Circulating miRNA as Predictive Tools for Immune Reconstitution Inflammatory Syndrome in HIV/TB Co-infected Individuals: A Proof-of-Concept Study

MicroRNAs (miRNAs) regulate post-translational gene expression and are potential biomarkers for infectious diseases. Immune reconstitution inflammatory syndrome (IRIS), a severe inflammatory response after antiretroviral therapy (ART) initiation, occurs as paradoxical IRIS (worsening of treated infections) or unmasking IRIS (response to undiagnosed infections). In the ANRS 12358 study (manuscript in preparation), we identified hsa-miR-146a-5p, hsa-miR-29c-3p, and hsa-miR-29a-3p as predictive IRIS biomarkers using a flow cytometry-based approach (Patent No: NT/NG/IDM-22-0055). This study further validates circulating miRNAs (n=27 miRs) as IRIS biomarkers in HIV/TB co-infected individuals. Plasma samples (n=660) from the CAMELIA trial and CAPRI-NK study in Cambodia were analyzed using the FirePlex™ miRSelect assay.

Statistical analyses identified four significantly differentially expressed miRNAs linked to inflammation, cytokine regulation, and NK cell activation, enhancing IRIS prediction. Combinatorial analysis improved accuracy, reducing false positives and negatives. However, technical challenges with a new version of FirePlex kit (e.g., buffer composition, probe concentration) led to over-saturation in miRNAs detection, preventing replication. Discontinuation of the original kit (e.g. the version of kit that we used in ANRS12358 study) further hindered validation. Despite these limitations, circulating miRNAs remain promising IRIS biomarkers, emphasizing the need for standardized detection methods. Further research is essential for clinical translation.

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

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

Additional tools are urgently needed not only to help diagnose TB but also to assess the response to TB treatment in empirically treated patients. In a previous study, we found that Interleukin-1 receptor antagonist (IL-1Ra) plasma concentrations dropped dramatically after two months of TB treatment. The objective of this proof-of-concept study is to demonstrate that IL-1Ra concentrations significantly decrease earlier within two weeks following TB treatment initiation in adults with documented TB.

In parallel, we are assessing two other biomarkers: Interferon gamma induced protein -10 (IP-10) and sCD163. The plasma of 22 TB+HIV- and 6 HIV+TB patients at day 0, week 1, week 2, week 4 and week 8 after anti-tuberculosis drugs treatment were analyzed. We have confirmed a significant decrease in IL-1Ra and IP 10 levels at week two. Interestingly, the decrease in IL-1Ra and IP 10 levels from week one after the treatment was similar. The study has published in the Journal of International Journal of Infectious Diseases, Volume 145, August 2024; DOI: 10.1016/j.ijid.2024.107096 .

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

Axis 3: Vaccine Responses to Rabies Virus Vaccination

The World Health Organization endorsed a new shortened protocol of post exposure prophylaxis (PEP) in the April 2018 guidelines. This *"Institut Pasteur du Cambodge* protocol" of three PEP sessions of two-site intradermal 0,1 mL vaccine doses each at days 0, 3 and 7 is the first one-week PEP regimen to be recommended (Cantaert T, Borand L et al, Lancet Infect dis 2019). The "IPC protocol" is to date the shortest and most vaccine-sparing rabies PEP protocol approved by the WHO.

In a follow up study we assessed one-year humoral and T cell immunity in PEP recipients of the Insitut Pasteur du Cambodge (IPC) regimen, recommended by WHO. We analyzed rabies virus (RABV) neutralizing antibodies (nAbs) and T cell responses at baseline, 7 and 14 days, 6 and 12 months after PEP. This study demonstrates robust one-year immunity after IPC PEP (Ya and Auerswald, NPJ Vaccines, 2024).

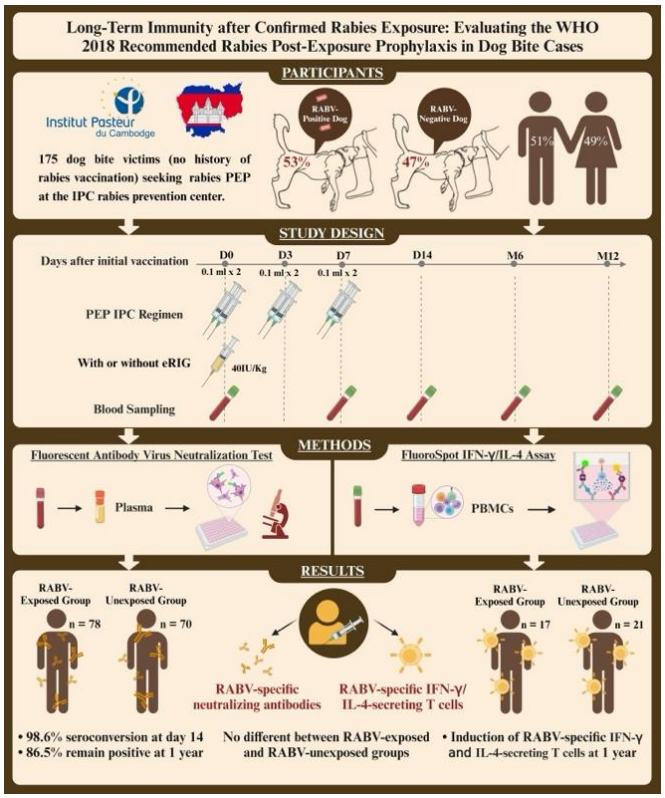


Figure 7. Evaluation of one-year immunity after rabies PEP in a Cambodian population. Ya and Auerswald, NPJ vaccines, 2024

Start/End Year	2019-2025
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

3.3.3 Research Programs – Outlook for 2025

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

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

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

A/ Assessment of dengue E-protein epitope-specific antibodies and correlation with disease severity. The Envelope (E) protein of DENV is known as the major target of antibody (Ab) response. Anti-Envelope domain III Abs (anti-EDIII Ab) are mainly serotype-specific, whereas anti-fusion loop (FL) Abs can induce antibody dependent enhancement (ADE) in vitro. Abs targeting the E-dimer epitope (EDE) are potent and broadly neutralizing against all four dengue serotypes. Resolving the complex

polyclonal response to E protein might uncover novel correlates of protection after infection. Therefore, we have set up a new assay to detect and quantify E protein-epitope specific Abs in plasma obtained from DENV infected humans by multiplex immunoassay (MIA) (Lay et al, submitted). In 2025, we aim to correlate levels of anti-EDIII, anti-E dimer and anti-fusion loop antibodies with disease outcome after dengue infection and follow the development of these antibodies over time.

B/ Identification of novel E-dimer specific antibodies

We aim to discover new anti-dengue antibodies with high cross-neutralization potential for dengue. We have established 2 pipelines for the discovery of novel potent anti-dengue antibodies (Figure 8). Given the low frequency of antigen-specific B cells and the high cost of the scRNAseq, we have optimized a patient-barcoded approach, which allows to pool multiple individuals before the 10x single cell analysis pipeline. B-cell receptor sequences showing a high clonal expansion profile will be selected for small-scale in-house mAb production to dengue E-dimer antigens. mAbs will be further characterized for breath, potency and effector function capacity. Secondly, we have established an additional mAb production pipeline involving single B cell cultures to enhance the efficient down-selection of highly functional mAbs. Here, single E-dimer-specific B cells are sorted and cultured. Supernatants will be screened for IgG production, E-dimer binding and functionality. Strong serotype cross-neutralizers will be selected for in house recombinant mAb production and further characterization. Stabilized E-dimer antigens are obtained from F.Rey and G. Barba-Spaeth from Institut Pasteur Paris.

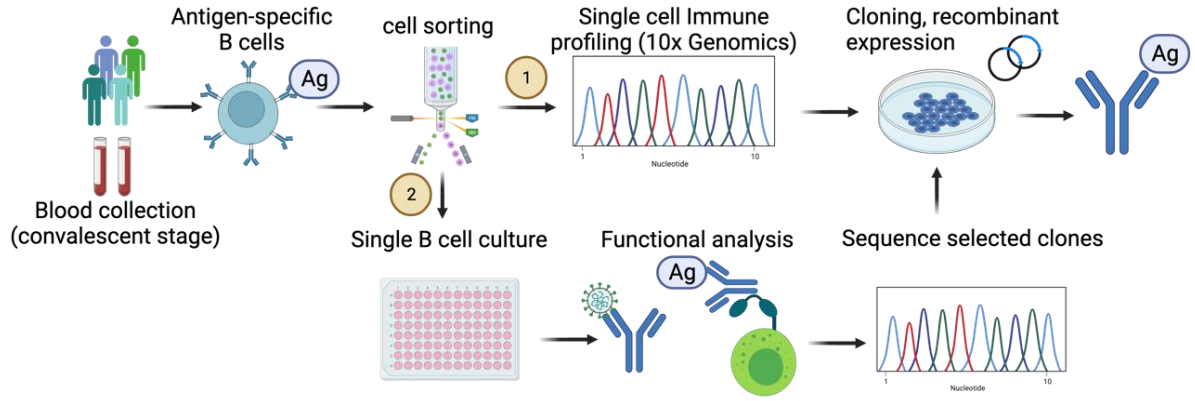


Figure 8: experimental pipeline for the production of mAbs.

Start/End Year	2020-2025
Collaborations	Virology Unit, IP Cambodia (DUONG V), Epidemiology and Public Health Unit, IP Cambodia (LY S), Institut Pasteur Paris (BARBA-SPAETH G, REY F, SAKUNTABHAI A, SIMON-LORIERE E), Antwerp University (ARIEN K)
Funding	NIH PICREID (1U01AI151758 – 01)

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

Other than virus neutralization, many other functions are attributed to antigen-specific antibodies in protection against viral infections, which are critically dependent on the formation of immune complexes, the Fc portion of IgG and interaction with Fcγ receptors and downstream effector functions. We have recently shown that DENV infection causes a specific increase in afucosylated IgG glycoforms, which correlates with disease severity and has prognostic potential (Bournazos et al, Science 2021). Hence, we identified a key role for the Fc glycan structure in dengue pathogenesis, but

the mechanism underlying this observation remains to be determined. Different IgG Fc glycoforms have different affinities for Fc gamma receptors, which activate and initiate downstream effector functions. One of these mechanisms crucially dependent on IgG Fc-Fc gamma receptor interactions is antibody-dependent enhancement (ADE). Therefore, we seeks to investigate how DENV infection modulates the glycosylation profile of IgG Fc and how IgG Fc-Fc gamma receptor interactions contribute to disease outcome after dengue infection. DENV could modulate the IgG1 glycosylation profile either by eliciting distinctive inflammatory cues to B cells or through direct infection of B cells. Dynamic changes in glycoenzyme expression over time will be assessed and in vitro conditions and pathways that lead to changes in glycoenzyme expression will be identified. Four *in vitro* cell-based assays have been optimized in the immunology unit to evaluate antibody-effector functions of IgG generated during dengue infection: antibody-dependent enhancement assay, antibody dependent cytotoxicity assay, antibody dependent phagocytosis assays, and complement dependent cytotoxicity. All four assay results will be correlated to clinical outcome and other parameters such as viral load, DENV IgG titers, platelet count, haematocrit and duration and severity of symptomatic infection. Protection and risk signatures will be uncovered using multivariate analysis methods.

Start/End Year	2023-2028
Collaborations	Virology Unit, IP Cambodia (DUONG V), Rockefeller University (RAVETCH J, BOURNAZOS S)
Funding	NIH R01

Investigation of the impact of lipid metabolism on dengue infection and pathogenesis

The geographic expansion of Aedes mosquitoes has led to an increase in Dengue worldwide. The only licensed vaccine has poor efficacy in dengue-naïve individuals and children. Efforts to combat this disease require a better understanding of the pathophysiological mechanisms involved in the transition to severe forms of dengue. METABODEN is a multidisciplinary project that will explore the interaction between disease severity and host lipid metabolism by studying the dengue virus (DENV)-induced lipid metabolism reprogramming and consequences in pathogenesis. METABODEN aims to (i) identify changes in lipid molecular species and key lipid metabolism enzymes in longitudinal pediatric samples in order to establish a link between DENV-induced blood lipid metabolic imprinting and disease severity and (ii) to decrypt and investigate on molecular mechanisms accounting for DENV-induced lipidome remodeling Besides increasing fundamental knowledge on the role of lipid metabolism in DENV pathogenesis, METABODEN will also lead to the identification of biomarkers for dengue severity and potential targets for antiviral intervention.

Start/End Year	2024-4046
Collaborations	Dorothee MISSE (IRD), Laurence BRIANT (IRIM), Fabien Blanchet (IRIM)
Funding	ANR

1.2. Immunity to *Aedes* mosquito saliva

To extend our observations in humans (see above, major achievements), we aim to investigate the effect of mosquito saliva on the immune response to dengue. We continue our collaboration with the LMVR, NIAID, NIH to further assess the immune responses to mosquito saliva in the skin of Cambodian individuals with a new clinical trial design.

Start/End Year	2023-2026
Collaborations	Laboratory of Malaria and Vector Research, NIAID, NIH (OLIVEIRA F)

	ICER Cambodia (YEK C)
Funding	NIH core funding

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

Plasmodium vivax (Pv) is the most widespread human malaria parasite and is particularly resilient to current elimination efforts. Naturally acquired immunity to Pv has been observed, where exposed subjects can develop partial immunity after multiple exposures to Pv enabling the control of parasite densities. However, life-long exposure rarely seems to confer sterile immunity and individuals can carry low-level parasitemia. Increased understanding of the immunological mechanisms, potential antigenic targets and parasite factors that confer protection to clinical Pv malaria is urgently needed for future vaccine development. Leveraging a longitudinal cohort we have constituted in endemic area of Cambodia in collaboration with the Malaria Molecular Epidemiology Unit, we have identified individuals displaying remarkable clinical protection against Pv. Firstly, we aim to understand the mechanisms driving the development of Blabs against PvDBP in humans. Phenotype, gene expression profile and B cell receptor (BCR) characteristics of DBP-specific memory B cell subsets will be assessed by flow cytometry and combining single-cell RNA and BCR sequencing in naturally infected participants with characterized amounts of BIabs. DBP-specific CD4+ T subsets will be interrogated for function and phenotyped by flow cytometry. Secondly, we aim to identify host immune factors associated to protection from clinical P vivax malaria independent of Blab development. To have a better understanding of the immunological factors associated to protection from Pv, we will study the single cell landscape of the immune response in individuals displaying various levels of protection against Pv malaria. Moreover, functional *in vitro* studies using sorted primary cell populations from these individuals will be employed to assess the function of monocytes, T and B cell subsets in Pv infection.

Start/End Year	2023-2028
Collaborations	Malaria Molecular Epidemiology Unit, IP Cambodia (POPOVICI J) University of Maryland (SERRE G)
Funding	NIH – R01

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

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

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

1.5. Characterization of the immunological mechanisms that drive chronic chikungunya disease pathogenesis

Chikungunya virus (CHIKV) represents a widespread mosquito-transmitted, arthritogenic virus that causes chronic debilitating joint pain and arthritis in almost half of infected patients. Currently, no effective therapies or biomarkers predicting chronic disease exist, as the mechanisms driving chronic CHIKV symptoms remain elusive. The central hypothesis of our proposal is that dysregulated antibody responses modulate susceptibility to chronic chikungunya through impaired antiviral activity, as well as excessive and inappropriate activation of pro-inflammatory pathways. This hypothesis will be tested by the in-depth characterization of a unique cohort of chikungunya patients with resolved or chronic disease. We will comprehensively analyze the immune responses during the acute phase of CHIKV infection, aiming to identify the immune determinants of susceptibility to chronic chikungunya disease. We will additionally characterize the heterogeneity of humoral immune responses from patients with differential disease outcomes. Lastly, using novel mouse models of chikungyunya disease, we will investigate the immunopathogenic mechanisms of CHIKV infection, as well as determine the mechanisms by which antibodies modulate disease pathogenesis. These studies are expected to lead to the identification of the immune pathways and biomarkers that are associated with disease chronicity, guiding the development of novel vaccination and therapeutic strategies to prevent or treat chikungunya disease.

Start/End Year	2024-2030
Collaborations	Rockefeller University (RAVETCH J, BOURNAZOS S)
Funding	Wellcome Trust Discovery Award

1.6. Pasteur International Unit

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

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

Axis 2: Biomarkers of Infectious Diseases

Aim 2.1 Fluorescent staining of Mycobacterium Tuberculosis antigens in blood monocytes as a Point-of-Care Assay for diagnosis of tuberculosis

Tuberculosis (TB) remains a major global health challenge, with over 10.8 million new cases annually, including drug-resistant strains. Early and accurate diagnosis is essential but imped by limitations of current methods like GeneXpert and acid-fast bacilli (AFB) staining microscopy, which require specialized equipment, sputum samples, and time-consuming, making them difficult to implement in resource-limited settings. Lipoarabinomannan (LAM), a Mycobacterium tuberculosis cell wall glycolipid, is a promising biomarker detectable in blood and urine. However, existing LAM-based tests (AlereLAM, FujiLAM) have low sensitivity, particularly in immunosuppressed patients, necessitating a more effective, rapid, non-sputum-based diagnostic tool. Preliminary findings indicate that LAM carriage monocytes can be detected using flow cytometry. This method offers higher sensitivity and faster results than conventional TB diagnostics. The study will aim to develop a point-of-care diagnostic tool to enhance TB detection where current methods are less effective. The study will conduct in TB-infected patients from Cambodia and Mexico to validate their diagnostic potential. This approach may help to improve TB screening, reduce diagnostic delays, and enhance patient outcomes. The study awarded by ImmunoTool *FlowISiam* 2025 and will submit to ARNS-MIE for grant support for patients enrollment sampling.

Start/End Year	2024-2025
Collaborations	IP Paris (SCOTT D), IP Cambodia (PEAN P), IP Cambodia (CANTAERT T); Leslie Chàvez-Galàn (Mexico); and Rogelio Hernàndez-Pado (Mexico)
Funding	ImmunoToll FlowISiam award 2025 (reagent's part).

Aim 2.2 Landscape and determinants of post-TB lung disease in people with and without HIV

Post-tuberculosis lung disease (PTLD) is a major but underrecognized cause of long-term morbidity, affecting up to 50% of TB survivors, particularly in low- and middle-income countries (LMICs). Cambodia, a high TB-burden country, lacks PTLD data and surveillance. HIV co-infection further complicates PTLD risk, with unclear effects of immune reconstitution inflammatory syndrome (TB-IRIS). The aims of the study are (1) to characterize PTLD epidemiology in Cambodia; (2) to assess the long-term impact of HIV and TB-IRIS on PTLD and (3) to identify immunological and inflammatory predictors of PTLD in adults with and without HIV. This study will provide the first systematic data on PTLD in Cambodia, enhance understanding of HIV-TB interactions in lung disease, and identify biomarkers for clinical interventions. The proposal was submitted to the NIH-NIAID on January 2025 for funding.

Start/End Year	t.b.d
Collaborations	University of Washington (Adrienne Shapiro); Harvard Medical School-Boston (Anne Goldfeld); Pean Polidy (IPC); Tineke Cantaert (IPC); Sam Sophan (Cambodian Health Committee)
Funding	Submitted to NIH/NIAID (January 2025)

Axis 3: Vaccine Responses to Rabies Virus Vaccination

We aim to continue our long-term follow up of individuals bitten by rabid suspected dogs receiving the IPC PEP regimen. Until 2026, individuals will reach a 5-year follow up after rabies PEP. Rabies virus neutralizing antibodies and rabies-specific T cells will be determined and compared at D0, Y2, Y3 and Y5. From these data, waning of immune-mediated protection (both humoral and cellular) will be determined.

Start/End Year	2024-2026
Collaborations	Virology Unit, IP Cambodia (DUONG V), Epidemiology and Public Health Unit, IP Cambodia (FLAMAND C, LY S), Vaccination center, IP Cambodia (PENG Y)
Funding	French ministry of foreign affairs and French embassy in Cambodia (FEF)

Course "Immune responses to arbovirus infections from a One Health perspective"

Most arboviral diseases cause a disturbance in the host immune response which leads to immunopathology. Therefore, knowledge on immune mechanisms induced by arboviruses and how these mechanisms are disturbed is very important to advance vaccine development. Hence, a course that expands and re-enforces knowledge in aspiring scientists in Asia on the immune responses to arboviruses, host-pathogen interactions, the animal models and human challenge models available is of crucial importance. In addition, recent advances in our understanding of the vector susceptibility to arbovirus infection, vector competence and vector-virus interactions will provide additional insight into this complex topic from a One health Perspective. We plan to organize an in depth immunology course focused on the immune response to arbovirus infections at the end of 2025. We will host a poster session, two skill development workshops and a mentoring sessions. This 6-day course will provide an in-depth exploration of the interactions between arboviruses, their vectors (mosquitoes, ticks), and vertebrate hosts. We will delve into host-pathogen interactions and the innate and adaptive immune responses to arboviruses. Understanding the immune responses to arbovirus infections is crucial for developing effective preventive and therapeutic strategies. The course will be held in the attractive city of Siem Reap, which is well connected by its international airport and is celebrated for its UNESCO world heritage site of Angkor Wat.

Start/End Year	2025
Collaborations	None
Funding	International Union of Immunological Societies – Pasteur Network

3.3.4 Support to National Authorities

Tineke CANTAERT, PhD and Polidy PEAN, MD, PhD are both coordinators of the immunology module used in master's degree year. The degree program is offered jointly by the (University of Health Sciences, Phnom Penh, Cambodia and *Universite Paris Saclay*, Paris, France).

3.3.5 Teaching and Training

Students

PhD students:

1. SANN Sotheary: University of Hasselt, Belgium (2019-2024). Visits to UHasselt are covered by a BOF/BILA grant of the Flemish Government
2. GUERRERO GOMEZ David: University of Montpellier, France (2020-2024)
3. LAY Sokchea: Antwerp University, Belgium (2023-2026)
4. RAULT Loeiza (based in New Caledonia): University of New Caledonia, France (2024-2028)

Internship/Master students:

1. Ylenia VAN DER SANDE: University of Antwerp. M2 Master thesis. "*Identification of the stimuli altering IgG glycosylation during dengue virus infection*"
2. Mary CHHIM: University of Health Sciences/University Paris Saclay. M2 Master thesis. "*Assessment of dengue antibody effector functions*"

3. sreyneat RUN: Royal University of Phnom Penh. Bachelor thesis. "*The Evaluation of Long-term T cell Responses to Rabies Vaccination In Patients Receiving the WHO 2018 Recommended Rabies Post-Exposure Prophylaxis (PEP)"*

Teaching

1. Tineke CANTAERT, PhD and Polidy Pean, MD, PhD: Master Infectious Diseases. University Paris-Saclay-University of Health Sciences, 10 hours (M1 and M2)
2. Tineke CANTAERT, PhD and Polidy PEAN, PD, PhD: Member of the Steering Committee International Master Infectious Diseases and coordinator of the Immunology Module (M1).
3. Tineke CANTAERT, PhD: Master Immunology-ImmunoPathology, Sorbonne University, 2 hours (M2)
4. Tineke CANTAERT, PhD, Master Vaccinology, University de Lyon, 2 hours (M2)
5. MOOC "patient Immunomonitoring", Institute of Tropical Medicine, Antwerp. 2 hours.

3.3.6 Outlook for upcoming 3 – 5 years

The strengths of the immunology unit in infectious diseases research are dependent on the commitment and quality of our workforce, our resources (e.g., biobanking, state of the art technology-including maintenance contracts, biosafety level II laboratories), strong collaborations with most other IPC Units (Virology, Epidemiology and Public Health, Medical and Veterinary Entomology, BAIA, Malaria Molecular Epidemiology Unit) and longstanding collaborations with excellent research groups worldwide. This is exemplified by our high-quality research output. We continue to emphasize collaborative work in an environment of cultural diversity. We aim to create an educational learning environment for students, PhD students and postdoctoral researchers with weekly lab meetings and monthly journal clubs.

Within the Pasteur network, we have formed a Pasteur International Unit with the Immunology Units of IP Madagascar and IP Dakar, respectively, to increase the visibility of Immunology in the Pasteur Network. In 2024, two innovative grant proposals have been awarded, a Wellcome Discovery Award (PI: Cantaert Tineke) aiming to understand the immunological mechanisms that drive chronic chikungunya disease pathogenesis, and a EU Horizon project (PI: Ali Mirazimi, Karolinska University) aiming to discover novel antibodies against Crimean Congo hemorrhagic fever surface glycoproteins. In addition, following research projects will be further conducted in the following years: 1/ R01 NIH (PI: Stylianos Bournazos, Rockefeller Univerity), on the investigation of IgG Fc- FcgR interactions during DENV infection, 2/ R01 NIH (PI: Cantaert Tineke and Popovici Jean) on the evaluation of immune responses in asymptomatic Pv-infected individuals, 3/ an ANR project (PI: Dorothee Misse, IRD) on the assessment of lipid metabolic changes during DENV infection, 4/ A collaboration with NIH researchers on the evaluation of skin immunity to mosquito saliva (PI: Fabiano Oliveira, NIH), 5/ Continue the follow up of a longitudinal PEP cohort after rabies exposure (PI: Claude Flamand).

3.3.7 Scientific Publications 2024

Note: *The name of authors from the Institut Pasteur du Cambodge are underlined*

1. Balancing functions of regulatory T cells in mosquito-borne viral infections

S. Sann, M. Kleinewietfeld, T. Cantaert

Emerging Microbes & Infections 2024, https://doi.org/10.1080/22221751.2024.2304061.

2. **Evaluation of one year immunity following rabies post-exposure prophylaxis in dog bite cases.**
   Ya N, Auerswald H, Touch S, In S, Yun C, Thai P, Sann S, Heng B, Leng C, Duong V, Peng YS, Ly S, Cantaert T. NPJ Vaccines. 2024 Nov 27;9(1):237. doi: 10.1038/s41541-024-01030-8.

3. **Increased frequencies of highly activated regulatory T cells skewed to a T helper 1-like phenotype with reduced suppressive capacity in dengue patients.**
   Sann S, Heng B, Vo HTM, Arroyo Hornero R, Lay S, Sorn S, Ken S, Ou TP, Laurent D, Yay C, Ly S, Dussart P, Duong V, Sakuntabhai A, Kleinewietfeld M, Cantaert T.
   mBio. 2024 May https://doi.org/10.1128/mbio.00063-24. PMID: 38752787

4. **In-vitro assessment of cutaneous immune responses to aedes mosquito salivary gland extract and dengue virus in Cambodian individuals**
   D. Guerrero, S. Lay, E. Piv, C. Chhin, S. Leng, R. Meng, K. E. Mam, P. Pean, A. Vantaux, S. Boyer, D. Missé, T. Cantaert
   Oxford Open Immunology, Volume 5, Issue 1, 2024, iqae003. Advance Access Publication Date: 1 April 2024 https://doi.org/10.1093/oxfimm/iqae003

5. **Rapid decrease in IL-1Ra and IP-10 plasma levels following tuberculosis treatment initiation**
   P. Polidy, R. Affi, C. Chazalon, B. C. Soumahoro, D. Gabillard, D. Bunnet, L.Borand, R. Moh, X.Anglaret, F.X Blanc, P.M Girard, G. Carcelain, D. Laureillard, L. Weiss
   International Journal of Infectious Diseases Available online 11 May 2024, 107096 https://doi.org/10.1016/j.ijid.2024.107096

6. **Unraveling the intricacies of host-pathogen interaction through single-cell genomics.**
   Gioacchino E, Vandelannoote K, Ruberto AA, Popovici J, Cantaert T.
   Microbes Infect. 2024 Feb 16:105313. doi: 10.1016/j.micinf.2024.105313.

7. **Zika virus T-cell based 704/DNA vaccine promotes protection from Zika virus infection in the absence of neutralizing antibodies.**
   Roth C, Pitard B, Levillayer L, Lay S, Vo HTM, Cantaert T, Sakuntabhai A.
   PLoS Negl Trop Dis. 2024 Oct 17;18(10):e0012601. doi: 10.1371/journal.pntd.0012601.

3.4 Virology Unit

3.4.1 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 of infectious diseases in Cambodia, in Southeast Asia, and around the globe. These activities comprise four main components, 1) arboviruses (e.g. dengue, Zika, chikungunya and Japanese encephalitis viruses), 2) respiratory syndromes (seasonal, avian influenza, COVID-19 and other respiratory viruses), 3) zoonotic and emerging pathogens (e.g. coronaviruses, hantavirus, Nipah virus and other emerging viruses), 4) Rabies and other viruses (enteroviruses, hepatitis viruses, etc.) The cross-cutting activities comprise of cell culture, virus isolation, sequencing, biosafety level-3 (BSL-3) laboratory, animal facility, quality, security and hygiene and administrative and stock management. The Unit is structured in three components: 1. Laboratory Management; 2. Technical Groups and 3. Research and Surveillance Teams (Figure 9).

In 2024, the unit comprises 45 staff including 2 Post-Doc, 5 PhDs, 2 PhD candidates, 7 master's degree holders, 1 medical doctor, 1 pharmacist and 27 technical staff. The ratio of female/male was 1.65 and there are 8 nationalities including 37 Cambodian team members. Within the research and surveillance groups, the Virology Unit has developed numerous research programs conducted in collaboration with other units at IPC—the Epidemiology and Public Health Unit (EPH), the Entomology Unit, and the Immunology Unit—as well as with governmental partners from the Ministry of Health (Cambodian CDC-MoH, National Center for Parasitology, Entomology and Malaria Control, National Institute of Public Health), the Ministry of Agriculture, Forestry and Fisheries (National Animal Health and Production Research Institute, General Directorate of Animal Health, Production and Forestry Administration), the Ministry of Environment (Department of Wildlife) and other collaborators across the globe. These programs focus on infectious diseases of concern to the Cambodian population.

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

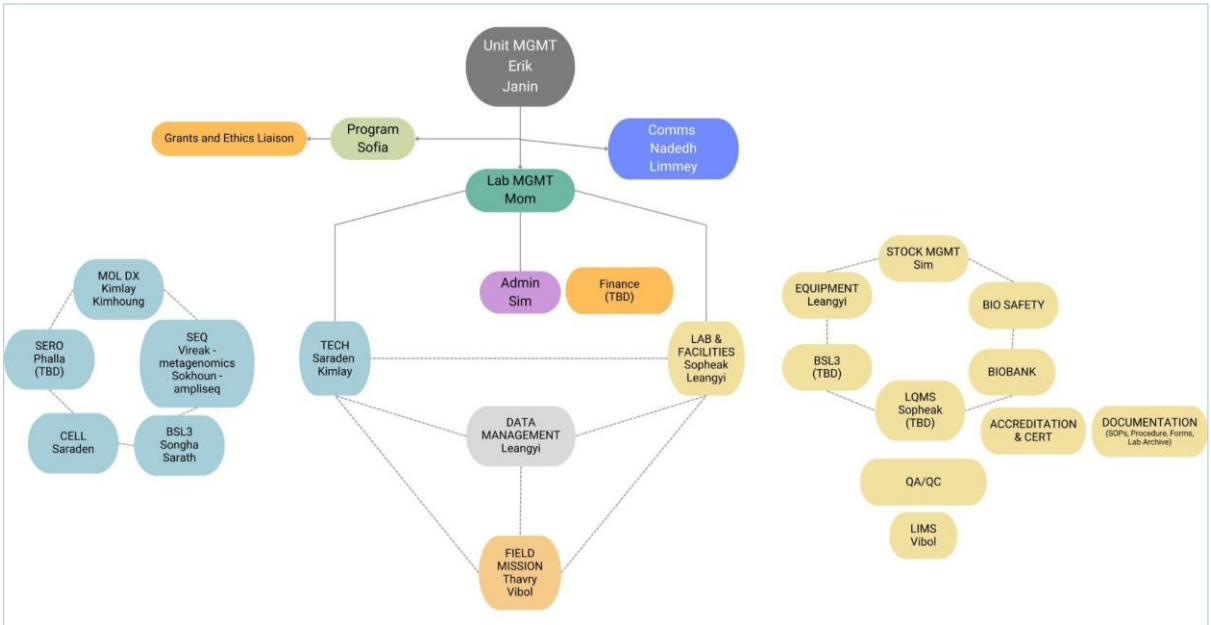


Figure 9: Virology Unit organigram, 2025

3.4.2 Research Programs – Major Achievements in 2024

Axis 1: Arboviral Disease

DenThom: Study of dengue-like illnesses in Kampong Thom Province, Cambodia.

The primary objective of this NIH-funded project within the CREID network is to study the prevalence and incidence of dengue and dengue-like illnesses in Cambodia by implementing a novel study site in Kampong Thom, a province in the center of Cambodia that is a major transport axis, and where information on dengue transmission and circulation is lacking so far. The full description of this project is detailed in the Epidemiology and Public Health Unit, the Immunology Unit and Medical & Veterinary Entomology Unit sections. 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. The inclusion started in April 2022. As of February 14, 2025, a total of 2,913 (860 in 2024) suspected dengue cases from the four hospitals were included, and 991 (221 in 2024) were positive for dengue virus (DENV) infection (RT-PCR confirmation). In 2024, the majority of samples came from Kampong Thom referral hospital (Figure 10). Among RT-PCR positive cases, Dengue virus serotype 2 was the predominant serotype accounted for 98.6% (218/221). For the household investigation around the index cases, we included 1,158 participants in 2024. Of these, 28 were PCR confirmed DENV positive, with DENV-2 as a predominant serotype accounting for 96.4% (27/28).

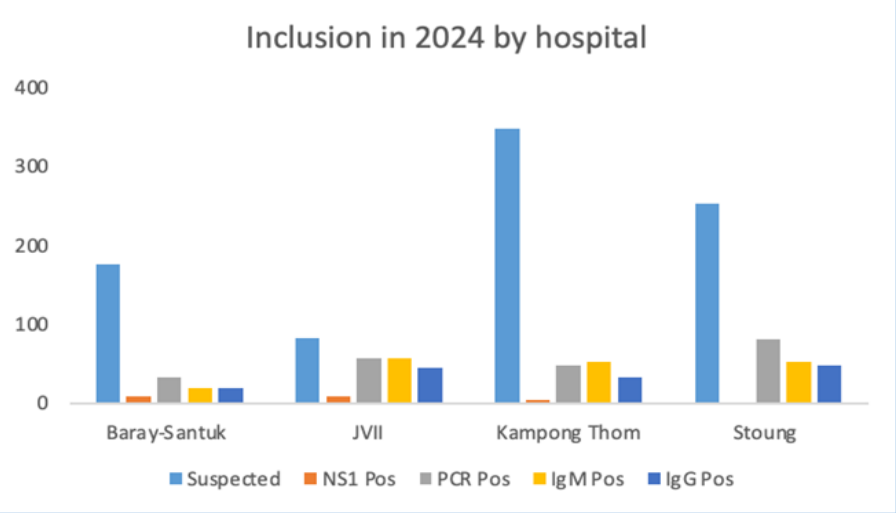


Figure 10: Number suspected cases from the four hospitals in 2024

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

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

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

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

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

Ticks transmit a greater variety of pathogenic agents than any other blood-feeding arthropods, posing a profound impact on the economy of livestock farming and human health concerns. Cambodia already has an enormously high burden of infectious diseases; however, little is known about potential hotspots of tick-borne pathogens. The diversity and distribution of tick species remain poorly characterized. The medical, veterinary, and socio-economic burdens of tick-borne diseases remain also largely unknown. In the present study, we aim to assess the diversity of tick species and associated pathogens circulating in a rural community of Cambodia and to evaluate the transmission risk of these pathogens in people living in the area where ticks have been collected.

Our study, conducted within the framework of DENTHOM project, took place in rural communities of Kampong Thom province located in the central part of Cambodia. Ticks were collected from animals and the vegetation surrounding 82 households. A total of 1,433 ticks were collected, primarily from dogs (97.3%). Among them, 91% were adult, dominated by Rhipicephalus sanguineus (97.3%) followed by Rhipicephalus microplus (2.4%) and Rhipicephalus haemaphysaloides (0.3%). Tick samples were screened for pathogens considered major threats to human health including severe fever with thrombocytopenia syndrome virus (SFTSV), tick-borne encephalitis virus (TBEV), and Crimean-Congo hemorrhagic fever virus (CCHFV), Rickettsia, Borrelia, Ehrlichia, and Coxiella using PCR assays. However, none of the targeted viruses were detected. To enhance the detection of tick-borne pathogens, 91 pools (15 – 16 ticks/pool) underwent metagenomics sequencing. Data analysis is currently in progress. To assess the transmission risk of targeted pathogens in human, we analyzed a subset of 200 serum samples collected from residents of tick affected households using commercial ELISA kits for the detection of IgG antibodies. Of these, 56% (111/200) tested positive for anti-TBEV IgG, 6% (11/180) for anti-SFTSV IgG, 3% (5/200) for anti-CCHFV IgG, 11% (21/200) for anti-scrub typhus

IgG, 5% (10/200) for anti-Borrelia, and 1% (1/180) for anti-Coxiella IgG. The evidence of past exposure to SFTSV was confirmed by sero-neutralization assay in 6 out of 11 ELISA positive samples, whereas no confirmation was observed for CCHFV. Similarly, exposure to TBEV was confirmed by a seroneutrilazation assay in 3 out of 10 samples strongly positive with ELISA.
Our study provided preliminary data on ticks and the potential risks of tick-borne pathogens circulating in the rural community of Cambodia. Large-scale studies implementing one health approach must be considered to better assess the threats of tick-borne diseases at the intersection of animal health, human health, and the environment in Cambodia.

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

Axis 2: Respiratory Viruses

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

In 2017, in collaboration with the Food and Agriculture Organization of the United Nations (FAO) and National Animal Health and Production Research Institute (NAHPRI), we sought to establish avian influenza virus surveillance in Cambodian border regions to obtain a greater understanding of the dynamics of cross-border movements of avian influenza viruses into Cambodia and to obtain molecular profiles of the circulating influenza viruses in Cambodia. During the 2018–2019 and 2020 study periods, we collected 5,120 poultry samples from 2,560 birds (paired tracheal and cloacal swabs). Overall, combining information from both tracheal and cloacal swabs on a “per bird” basis, avian influenza virus was detected in 27.8 % (n=712) of the bird sample. Surveillance resumed in fall 2021 (after some delays due to COVID-19). In 2023, this collection continued in the beginning and late parts of the year, with 3,600 samples collected from 1,800 birds, and 425 environmental samples. In 2024, the collection included 5,120 poultry samples (tracheal and cloacal samples) and 780 environmental samples (see below).
The collection, analysis, and sequencing are complete for the 2017–2023 sessions, 2024 is ongoing, and several manuscripts are in preparation (see below). This collaboration is planned to continue for the 2025 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 2024 are being sequenced and phylogenetic, antigenic, and molecular risk assessments are underway in collaboration with the WHOCC in Melbourne, the World Influenza Centre at the Francis Crick Institute in the United Kingdom, the US-CDC in Atlanta, USA, the University of Hong Kong, and other partners. Since 2021, IPC has worked with partners at the Johns Hopkins University's Applied Physics Laboratory to use a novel multi-segment, barcoded PCR for sequencing using Oxford Nanopore Technologies. We have successfully established this pipeline and are internally sequencing 24-48 influenza samples/week from AIV surveillance.

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

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

Recently, HPAI A/H5N1 viruses of clade 2.3.4.4b were detected in live bird market surveillance during Lunar New Year, 2023. Concerningly, A/H5N1 clade 2.3.4.4b viruses containing HA genes closely related to A/Astrakhan/3212/2020 have caused major devastation in poultry and wild birds globally, and numerous spillovers into mammalian hosts such as mink and marine mammals, and human infections in the UK, the USA, and Ecuador. In addition, HPAI A/H5N1 clade 2.3.2.1c viruses were still detected regularly into 2024 alongside these 2.3.4.4b viruses.

Upon deeper molecular and phylogenetic analysis of the genomes of the viruses from human cases and associated poultry samples from late 2023 into 2024, it appears that the currently circulating strain affecting both poultry and humans in Cambodia is a reassortant virus between clades 2.3.2.1c and 2.3.4.4b, some carying a PB2 627K mutation (see further details in rest of report). At the current time, it is not clear how, when or where the reassortment and mutation occurred. While HA gene of this virus clusters phylogenetically with clade 2.3.2.1c H5N1 strains that have circulated recently in Cambodia and Southeast Asia, the internal gene constellation of this reassortant makes it a novel viral lineage. In addition, at the current time, it is not clear how this reassortment and mutation affects risk of this virus to either poultry or humans. Each gene segment impacts viral phenotypes like transmissibility, host adaptation, and even drug resistance. Therefore, this reassorted genome represents more than a repackaging of existing Southeast and East Asian H5N1 genetics. Instead, it constitutes a new viral strain that warrants close monitoring and risk assessment even though the HA and NA continue to resemble regional strains from a phylogenetic perspective. As of 2024, this reassortant 2.3.2.1c virus has taken over detections in LBMs and is the dominant circulating genotype of 2.3.2.1c in Cambodia.

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 2020–2024 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 2025.

Continued detection of endemic A/H9 viruses in Cambodian live bird markets.

Subtype A/H9 AIVs circulate globally in wild avian species, and are endemic in domestic poultry in many Asian, Middle Eastern, and African countries. A/H9 AIVs also commonly donate internal protein genomic segments to non-A/H9 viruses through reassortment, increasing zoonotic potential. In 2013, surveillance efforts in Cambodia expanded to encompass A/H9 viruses. It is now evident that A/H9 LPAI viruses circulate endemically in Cambodian poultry similar to Bangladesh, China and Vietnam and LBM workers are exposed to these viruses. All A/H9 viruses identified so far in Cambodia are classified as having an N2 subtype NA and sequencing indicates all of these viruses fall into clade 4.2 (BJ94/Y280-like). The majority of viruses are similar to those circulating in Vietnam and Indonesia between 2014 and 2019. The majority of Cambodian A/H9N2 viruses detected after 2015 belong to two genotypes, P and V. Human infection with A/H9 is rare, but not unheard of. The first human infection with A(H9) virus was reported in Hong Kong in 1998. Infections have occurred mainly in China and Hong Kong; however, some cases have been reported in Bangladesh, Pakistan, Oman, Senegal, and Egypt. Since 1998, >80 A(H9) human infections have been documented. The majority of cases were children.

In 2022, 6 cases of avian influenza A(H9N2) were reported to WHO from China. In the Western Pacific region, 71 cases of human infection with avian influenza A(H9N2) including two deaths (both with underlying conditions) have been reported to WHO since December 2015. Cambodia detected 2 cases of A/H9N2, one in 2021 (published) and one in 2022 (paper expected mid-2025). A detailed report of A/H9N2 circulation and evolution within Cambodian LBMs is expected in mid- to late 2025.

Detection of rare avian influenza subtypes in Cambodian poultry.

AIV in Asia is a complex system of many subtypes and a highly porous wild bird-poultry interface. Certain AIV subtypes, such as H14, are underrepresented in current surveillance efforts, and remain poorly understood in terms of its ecology and evolution. In 2022, we detected a rare subtype H14 in Southeast Asia, a geographic location and domestic bird system not previously known to harbor this subtype. These H14 viruses have a complex evolutionary history across gene segments including reassortment events, with sequences similar to endemic AIVs in Cambodian ducks, Eurasian low pathogenic AIVs and Eurasian highly pathogenic H5Nx AIVs.

The detection of H14 in Southeast Asia further advances our knowledge of the ecology and evolution of this subtype and reinforces the need for future vigilance in longitudinal, active surveillance in domestic poultry and wild birds as well as *in vivo* and *in vitro* risk assessment to include rare AIV subtypes that could become endemic in poultry systems. The manuscript was published in Emerging Microbes & Infections.

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

Swine influenza viruses

While swine production in Cambodia was traditionally characterized by backyard, small-scale farming efforts, larger confinement farms have increased in number in recent years. Imported pigs (via both legal and illegal routes) from Vietnam and other surrounding countries are becoming more common. Overall, expansion of the swine industry in Cambodia, coupled with negligible biosecurity, and mixed farming of pigs and poultry create a major risk for human, swine, and avian influenza strains to mix and transmit.

To better understand (IAV) diversity, epidemiology, prevalence, and disease dynamics in Cambodia, virological surveillance in swine from backyard farms in Cambodia was undertaken in 2011–2013. Those studies found that 1.5 % of sampled pigs were positive for triple reassortant H3N2 viruses similar to human H3N2 viruses previously isolated in Southeast Asia. A/H1 and A/H3N2 swine lineages were detected as well as A/H5 and H9N2 in Cambodian swine, which is concerning for zoonotic potential. In 2020, swine samples were sent to collaborators at Duke University and the National University of Singapore (NUS) for attempts at full genome sequencing to understand the viruses detected in these animals. These analyses have been completed.

Further swine-related work was funded through NIAID-funded Centers for Excellence in Influenza Research and Response (CEIRR) for 2021–2028 and is under further consideration for funding from other sources. In 2021, we were successfully awarded project funding through UPenn's CEIRR/Royal Veterinary College (RVC), established swine and human surveillance, and began ethical clearance for the studies. Sampling and analysis commenced in 2022, with collection continuing into 2023. Sampling was ended in 2024 due to issues fully establishing the project and changes in funding. No influenza viruses have been detected in swine samples in 2024.

Research Project Name	Swine influenza viruses
Funding	US-DHHS, FAO, NIH/NIAID/CEIRR
Project Duration	2021-2024 (Project ended early in 2024)
Collaborations	Institut Pasteur du Cambodge: Virology Unit (E. Karlsson), EPH Unit (S. Ly, M. Chan) MoH/CCDC, NHPRI/GDP, FAO, WHO CC Melbourne/Peter Doherty Institute, Australia, Duke-NUS, WHO, RVC/UPenn, AAHL/CSIRO, London School of Tropical Medicine and Hygiene, UHS, CEIRRA

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

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

Attempts are being made to classify viruses of interest, especially the Respiratory Syncytial Virus (RSV), coronaviruses, and paramyxoviruses. Some samples were shipped to collaborators at Duke-NUS in Singapore for next-generation sequencing in late 2020. Phylogenetic analysis and sequencing of the 2021-2023 samples are ongoing. Analysis of the etiology database was completed in 2023 and we are actively collaborating with epidemiologists and biostatisticians for the best way to analyze and present the data. A manuscript describing the 2014–2023 comparative data between Severe Acute Respiratory Illness (SARI) cases and healthy children, as well as reasons for increased mortality from influenza between 2016 and 2017, as compared to 2014–2015 is in preparation and is projected to be submitted mid-2025.

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

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

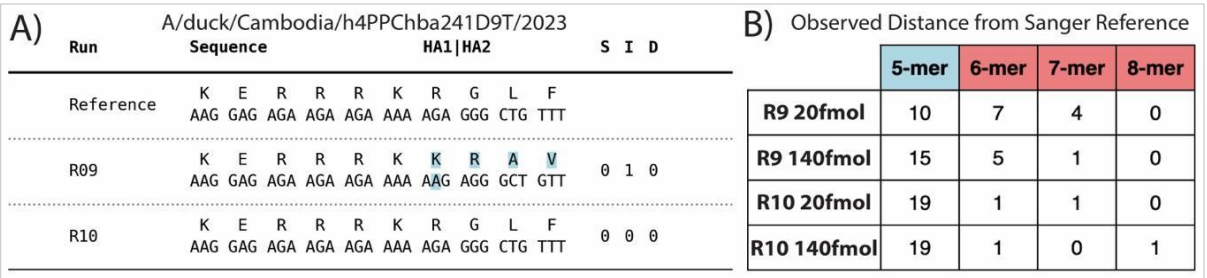
In Cambodia, current pathogen surveillance systems rely primarily on sampling and testing individual animals – a practice that is both costly and time-consuming, and prevents widespread coverage of all high-risk areas. One way to address this issue is the incorporation of environmental sampling (ES) into surveillance programs. ES includes samples or swabs taken from soil, water sources (drinking, carcass wash, lakes and ponds), feeding sources, feathers, air, and surfaces such as cages, chopping boards, and defeathering machines. As such, environmental pathogen surveillance casts a wide net at high-risk interfaces, potentially improving surveillance coverage and supporting expanded sampling on a longitudinal basis. We hypothesize that utilizing ES can: 1) improve, expand and simplify existing methods of pathogen surveillance; 2) reduce the cost of pathogen surveillance programs; 3) reduce direct contact between people and large numbers of animals, thereby improving biosafety, animal welfare, and reducing occupational exposure risks; and 4) set a precedent for lower-middle income countries (LMICs) to conduct broad pathogen surveillance cost-effectively. In 2024, we were able to collect 228 ES for comparison to individual bird samples. Sampling continues into 2025 and analysis is ongoing.

Research Project Name	Surveillance activities in Cambodia and using novel collection and sequencing techniques
Funding	Food and Agriculture Organization of the United Nations
Project duration	2022-2024

Collaboration	Institut Pasteur du Cambodge : Virology unit (E. Karlsson) Ministry of Agriculture, Forestry and Fisheries: National Animal Health and Production Research Institute

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

Rapid and accurate genomic surveillance is critical for monitoring viral mutations, tracing transmission, and guiding interventions in near real-time. Oxford Nanopore sequencing holds promise for real-time influenza genotyping, but data quality from R9 chemistry has limited its adoption due to challenges resolving low-complexity regions such as the biologically critical hemagglutinin cleavage site, a homopolymer of basic amino acids that distinguish highly pathogenic strains. In this study, human and avian influenza isolates (n=45) from Cambodia were sequenced using both R9.4.1 and R10.4.1 flow cells and chemistries to evaluate performance between approaches. Overall, R10.4.1 yielded increased data output with higher average quality compared to R9.4.1, producing improved consensus sequences using a reference-based bioinformatics approach. R10.4.1 had significantly lower minor population insertion and deletion frequencies, driven by improved performance in low sequence complexity regions prone to insertion and deletion errors, such as homopolymers. Within the hemagglutinin cleavage site, R10.4.1 resolved the correct motif in 90% of genomes compared to only 60% with R9.4.1. Further examination showed reduced frameshift mutations in consensus sequences generated with R10.4.1 that could result in incorrectly classified virulence (Figure 15). Improved consensus genome quality from nanopore sequencing approaches, especially across biologically important low-complexity regions, is critical to reduce subjective hand-curation and will improve local and global genomic surveillance responses. Paper is has been published in *Microbiology Spectrum*.



B) Observed Distance from Sanger Reference

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

Figure 11: Improved Resolution of the H5 Multi-Basic Cleavage Site. (A) Representative multiple sequence alignment for HA segment inclusive of the multibasic cleavage motif, composed of Sanger (Reference), R9, and R10 consensus outputs. 20fmol and 140fmol consensus outputs were identical for both R9 and R10 chemistries for this sample. R9 output includes a single base insertion at the 3' end of the adenine homopolymer upstream of the RGLF motif, resulting in an apparent frameshift mutation (highlighted in blue). S = # of substitutions / I = # of insertions / D = # of deletions. (B) Length of adenine homopolymer in consensus outputs for both chemistries and loading concentrations for all high pathogenicity avian influenza samples (n = 21). Lengths greater than five bases are assumed to be artifactual insertions.

Research Project Name	Improving Resolution of Highly Pathogenic Avian Influenza Virus Haemagglutinin Cleavage Site Using Oxford Nanopore R10 Sequencing Chemistry
Funding	WHO, Internal Funding
Project duration	2023 - 2024
Collaboration	John's Hopkins Applied Physics Laboratory

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

The project began in 2024 with the objective of investigating avian influenza virus (AIV) transmission dynamics in Cambodian live bird market (LBM) networks. Live bird markets present ideal conditions for the emergence and spread of AIV due to high bird densities and complex trade interactions. Understanding and controlling these dynamics is crucial to prevent outbreaks and reduce the risk of human infection.

Planned activities initiated in November 2024 include:

1. **Cross-Sectional Study (Nov 2024 - Feb 2025):** Conducted during high-risk periods coinciding with major festivals such as Bon Om Touk, Lunar New Year, and Khmer New Year. The study involves multi-stage sampling of LBMs and farms primarily in provinces with previous outbreaks and high poultry density, such as Battambang, Kampong Thom, Takeo, Prey Veng, Svay Rieng, and Phnom Penh. Structured interviews using Open Data Kit (ODK) on tablets target traders and middlemen to collect detailed information on trading, biosecurity, and production practices. Approximately 100 premises are expected to be visited, with around 65 samples collected per site (bird and environmental samples).
2. **Longitudinal Study (Planned for Nov 2025 - Feb 2026):** Building upon findings from the cross-sectional study, this phase will regularly monitor key LBM networks identified as high-risk. At least 20 premises will be visited on a routine basis (weekly, bi-weekly, or monthly), gathering ongoing data on trading behaviors, biosecurity measures, and sample collection to monitor AIV circulation and spread.

Through these combined efforts, the project aims to identify network connectivity factors, understand AIV emergence and spread patterns, and develop effective surveillance and control recommendations to mitigate future outbreaks.

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

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

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

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

In 2024, we successfully initiated this collaborative project, specifically focusing on recent human cases of H5 influenza occurring between 2023 and 2024. Our consortium performed an extensive

retrospective analysis of all available H5 strains collected from 2006 through 2024, significantly enhancing understanding of the genetic evolution, transmission dynamics, and epidemiology of highly pathogenic avian influenza (HPAI) H5N1 viruses circulating in the region. The initial findings and comprehensive analysis from 2024 are summarized in a scientific paper currently under review at the New England Journal of Medicine.

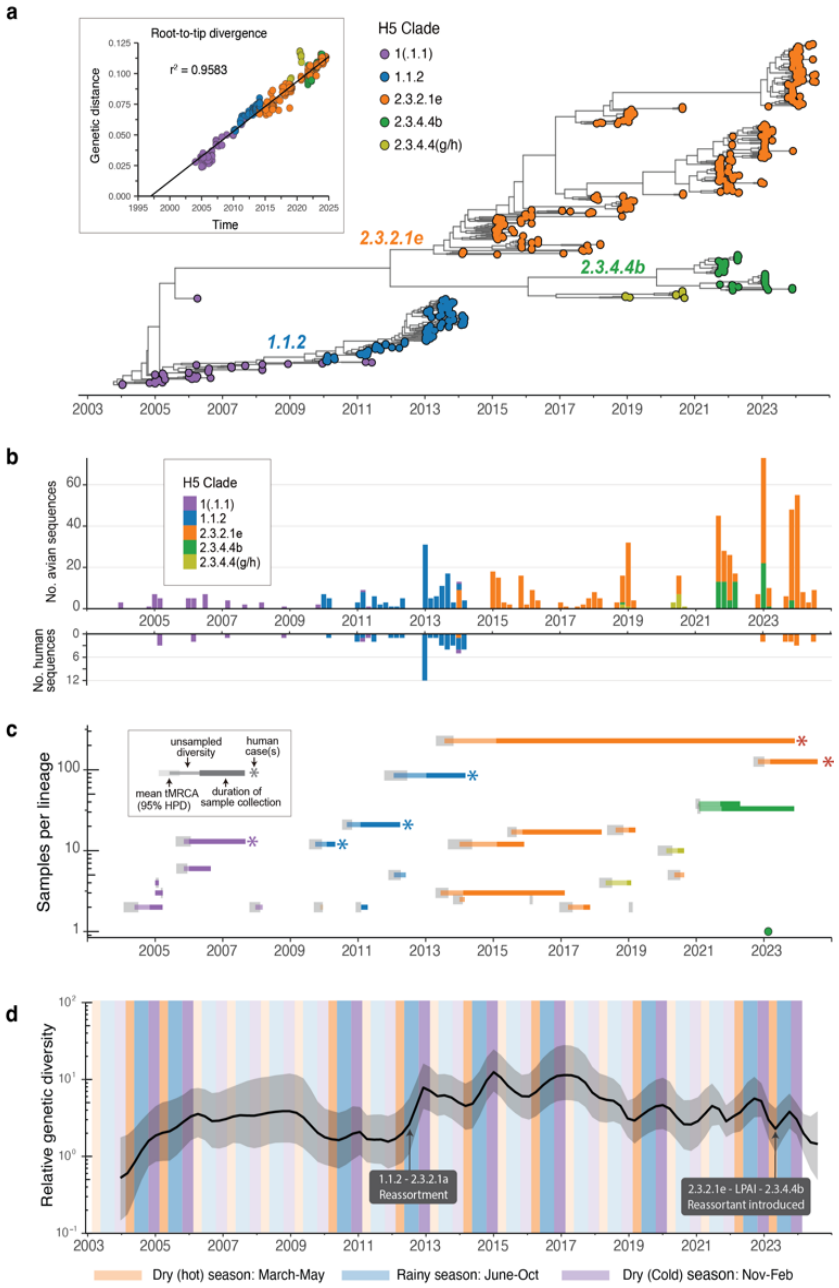


Figure 12. Long-term evolutionary dynamics of HPAI H5 viruses in Cambodia. a) Maximum clade credibility tree of HA gene sequences from Cambodia, with tip colors indicating clade assignment. The inset shows root-to-tip regression of genetic distance (y-axis) and sample collection dates (x-axis). b) Number of avian (top) and human (bottom) HPAI H5 viruses sequenced in Cambodia. c) H5 HA linage sizes and inferred durations of lineage persistence, based on a time-scaled maximum-likelihood phylogeny of all available H5 HA sequences (N = 24,382). d) Relative genetic diversity of HA gene, estimated using a coalescent-based GMRF Bayesian Skyride model. Major reassortment events and lineage introductions are annotated.

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

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

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

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

Axis 3: Zoonoses

Rodents as reservoirs for Hepatitis E virus (HEV), Arenavirus and other rodent-borne viruses and Risk Assessment of Infection in Human in Cambodia – HEPAR project

Rodent-borne viruses, including hantaviruses, arenaviruses, and rodent hepatitis virus (HEV-C), pose significant health threats to humans, causing severe diseases such as hemorrhagic fevers, respiratory illness, and hepatitis. In Cambodia, data on these viruses remain limited. This study investigated the presences of these viruses in rodents and assessed potential human exposure across diverse environmental and socio-economic contexts in Cambodia.

The study was conducted in urban, semi-urban, and rural areas of Cambodia during the rainy (2020) and dry seasons (2022). Rodents were screened for arenavirus, hantavirus, and HEV-C using RT-PCR. Human serum samples from the same site were tested for IgG antibodies using ELISA. Factors associated with virus spillover into humans were analyzed.

Among 750 rodents, 9·7% carried at least one virus: 5·2% arenavirus, 3·3% hantavirus, and 1·9% HEV-C. Infection rates were highest in urban (14·5%), followed by semi-urban (11·9%) and rural (2·1%) interfaces. Arenavirus was more prevalent during the rainy season, while hantavirus and HEV-C remained consistent across seasons. Seroprevalence in human was 12·7% for arenavirus, 10·0% for hantavirus, and 24·2% for HEV, with higher rates associated with urban residency and lower education level, prior medical conditions, flood-prone living area. Our findings highlighted the need for rodent control, improved market infrastructure, enhanced waste management, and public awareness on hygiene practices and zoonotic risks, especially in urban and high-risk areas.

In 2024, we focussed on data analysis and manuscript preparation. As of Feb. 20, 2025, one manuscript has been submitted to *The Lancet Global Health* and the preprint version is available on *medRXiv* (https://doi.org/10.1101/2025.02.09.25321973). A second manuscript on characterization of Seoul orthohantavirus is under preparation.

Research Project Name	Rodents as reservoirs for Hepatitis E virus (HEV), Arenavirus and other rodent-borne viruses and Risk Assessment of Infection in Human in Cambodia - HEPAR
Funding	IPC Internal Funding and CREID Pilot Program
Project duration	2020 – 2022
Collaboration	Institut Pasteur du Cambodge: Virology Unit (J Guillebaud, J Nouhin, V Hul, T Hoem, O Yanneth, M Sim, L Khun, P Y, L Heng, S Ken, L Pum, R Lim, K Chel, S Nuon, S Hoem, EA Karlsson, P Dussart, V Duong); Epidemiology and Public Health Unit (K Nguon, M Chan, and S Ly) Ministry of Agriculture, Forestry and Fisheries (C Meng) Institut Pasteur Paris (J-M Reynes and A Sakunthabhai)

Characterization of rodent-associated Hepatitis E virus (HEV-C) in Phnom Penh, Cambodia

This is a sub-study of the Hepar project aiming to characterize HEV-C in rodents collected from different markets in Phnom Penh. In the Hepar project, 14 out of 750 rodents tested positive for HEV-C using RT-qPCR, all of which were *Rattus novegicus*. To obtain the genome of HEV-C potentially specific to Cambodian rodents, we conducted the sequence-independent, single-primer amplification (SISPA) metagenomics on rodent liver samples using Oxford Nanopore Technologies (ONT) GridION sequencer and Illumina NovaSeq Platform. SISPA metagenomic on the ONT platform yielded HEV-C sequences from 4 out 14 rodents, each covering more than 50% of the viral genome (Table 5).

Table 5: Virus genome coverage of HEV-C obtained from metagenomics on ONT platform

Sample ID	Virus Nucleotide Number	Genome breadth coverage (^{3}10X)	Depth coverage, median (IQR)
HAR0012	6,932	96%	1,230 X (550 X – 2,459 X)
HAR0030	3,934	55%	185 X (151 X – 449 X)
HAR0040	6, 316	88%	129 X (33 X – 239 X)
HAR0058	4,725	66%	53 X (30 X – 78 X)

The preliminary phylogentic analysis of HEV-C sequences of these 4 rodents revealed that these Cambodian HEV-C strains belong to HEV-C genotype 1, clustering within the subtype 1a (Figure 13). The analysis of sequences obtained from metagenomics on Illumina NovaSeq platform is still ongoing.

Figure 13: Phylogentic tree of HEV-C from Cambodian rodents collected in Phnom Penh, 2020-2022. The sequences were aligned with HEV-C representative genotype sequences described by Bai et al., 2020 (PMID: 32630296) using MAFFT v.7.490 (PMID: 23329690) and phylogenetic trees were constructed using FastTree (PMID: 19377059). Trees were visualized and annotated using FigTree v.1.4.4. Cambodian rodent HEV-C are indicated in red.

The analysis of sequences obtained from metagenomics on Illumina NovaSeq platform is still ongoing.

Research Project Name	Characterization of rodent-associated Hapatitis E virus (HEV-C) in Phnom Penh, Cambodia
Funding	IPC Internal Funding
Project duration	2023 – 2024
Collaboration	Institut Pasteur du Cambodge: Virology Unit (L Pum, B Nalikka, K Mae Bienes, EA Karlsson, V Duong, G Wong, J Nouhin)

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

The BCOMING project's main objective is to co-construct innovations with all stakeholders in biodiversity hotspots to reduce the risks of infectious disease emergence through biodiversity conservation and zoonotic disease surveillance. The activities of the project are implemented in Europe and three tropical biodiversity hotspots in Southeast Asia, West Africa and the Caribbean.

In Cambodia, the BCOMING project will help understanding and preventing the risk of emergence of coronaviruses (CoV) and paramyxoviruses. It will be implemented in three Provinces with different levels of anthropisation and biodiversity protection measures: Phnom Penh (urban environment), Battambang (intensive agriculture) and Stung Treng (fragmented forest and rural agriculture). Biological sample collection was initiated in 2023 with the longitudinal follow-up of bats population in three selected karst hills in Stung Treng province with concurrent sampling of bats individuals, ectoparasites, vectors (mosquitoes, sand flies) and environment specimens (bat guano, air, feces, soil, hair). Over nine missions between 2023 and 2024, 1,845 bats were captured, encompassing 11 genera

and 25 species. Rhinolophus species represented 61.7% (1,138/1,845) of the sample size, and R. shameli accounted for 87.8% (998/1,138). Laboratory analysis and further characterization are ongoing but already highlighted a co-circulation of SARS-CoV-2-related viruses in different Rhinolophus bat species. Animal data collection is ongoing and additional field works in other provinces encompassing concurrent sampling of wild (bats, rodents) and domestic (poultry) animals, as well as human population living nearby will start early 2025.

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

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

In Cambodia, current pathogen surveillance systems rely primarily on sampling and testing individual animals—a practice that is both costly and time consuming and prevents widespread coverage of all high-risk areas. One way to address this issue is the incorporation of environmental sampling (ES) into surveillance programs. ES includes samples or swabs taken from soil, water sources, feeding sources, feathers, and even the air. As such, environmental pathogen surveillance casts a wide net at high-risk interfaces, potentially improving surveillance coverage and supporting expanded sampling on a longitudinal basis. In addition, after collection, samples are currently with molecular diagnostics tested after transport back to a laboratory, another time-consuming process. Therefore, the incorporation of field-based detection methodology can greatly reduce the time requirements for pathogen detection and give greater ability to respond to zoonotic pathogens of concern. Comparison of air, water, and surface sampling, PCR detection techniques, and metagenomics for these samples is ongoing, and a publication is expected by mid-2025.

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

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

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

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

In 2024, we successfully demonstrated that environmental surveillance, specifically air sampling combined with targeted metagenomics, effectively captures circulating zoonotic viral diversity at poultry live-bird markets (LBMs) in Cambodia. The study revealed that environmental samples frequently outperformed traditional poultry swabs in detecting avian influenza viruses, including highly pathogenic strains such as Influenza A/H5N1 clades 2.3.4.4b and 2.3.2.1c. This approach provided broader pathogen detection capabilities, including over 40 additional viruses across significant avian pathogen families, enhancing our ability to monitor and respond rapidly to zoonotic threats. This innovative surveillance method has significant implications for improving pathogen detection, outbreak prevention, and pandemic preparedness, and our manuscript detailing these findings is currently under review at *Nature Communications*.

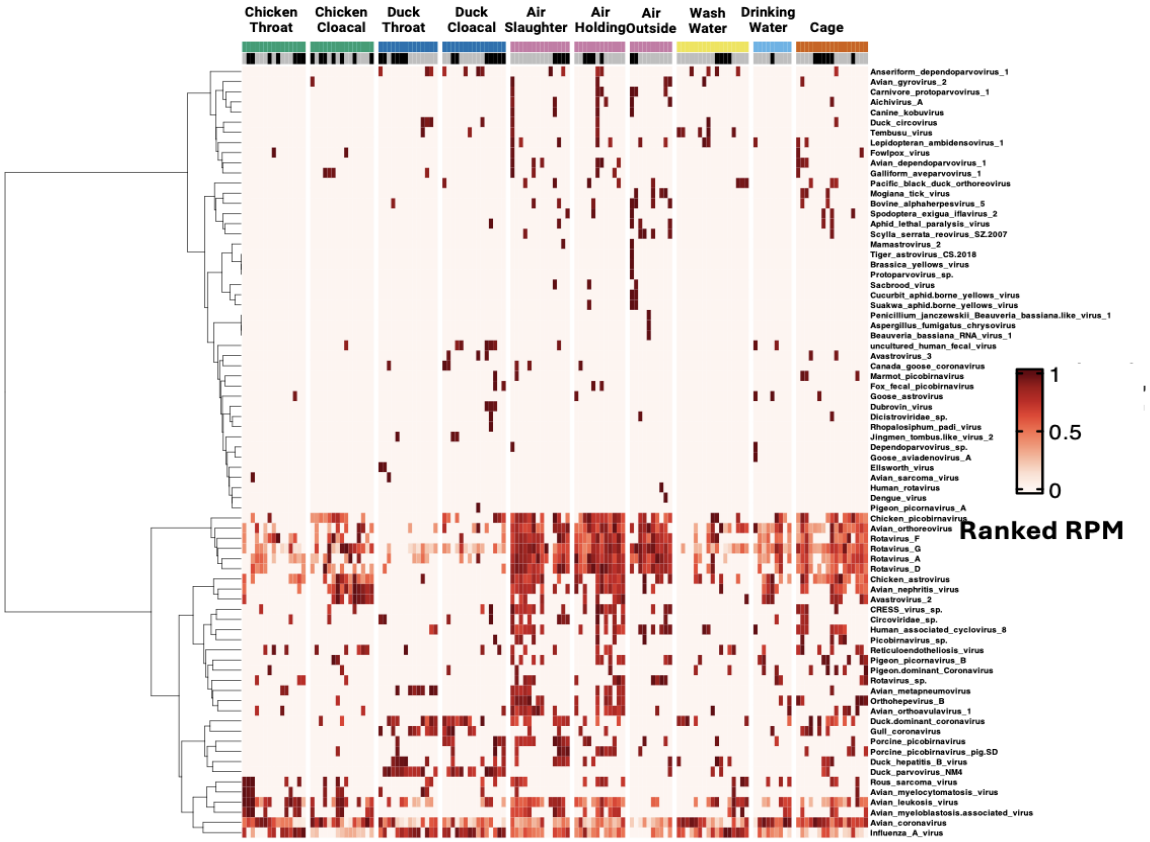


Figure 14: Comparison of air samples in LBM with other ES and individual duck and chicken samples

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

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

Backyard livestock farms, LBMs, and wildlife farms often have limited biosafety measures in place despite representing potentially risky human-livestock-wildlife interfaces, and as a result, have repeatedly been associated with the (re-)emergence of zoonotic diseases. Therefore, developing early warning systems at these high-risk locations will be critical for monitoring and preventing zoonotic diseases from generating the next pandemic. Previous pathogen surveillance and virus diversity studies at human-livestock-wildlife interfaces rely heavily on consensus time consuming and costly

methodology mostly focused on a single pathogen and/or species, and may result in delayed disease detection and reporting. Every second counts between first detection and response. Indeed, the total cost of an outbreak grows exponentially as time from detection increases, making identification critical at emergence or early stages of spread. It is critical to get "left of sneeze." To this end, the proposed project aims to evaluate family level consensus-based approaches for multiple viral pathogens on broad coverage samples coupled with real-time, in-field sequencing as a modern alternative to pathogen surveillance at high-risk interfaces.

In 2024, we developed and validated the Panviral Integrated Indexed Primers (PiiPs), significantly streamlining the detection of four major virus families: influenza, coronaviruses, flaviviruses, and lyssaviruses. The new PiiP assay improved multiplex PCR efficiency, reduced workflow steps from 22 to 12, and demonstrated robust performance in spiked and real biological samples without significant inhibition. Integration with the Basestack bioinformatics platform further enabled rapid, accurate, real-time pathogen detection and genotyping. Development of these novel primers and sequencing pipelines is ongoing into 2025.

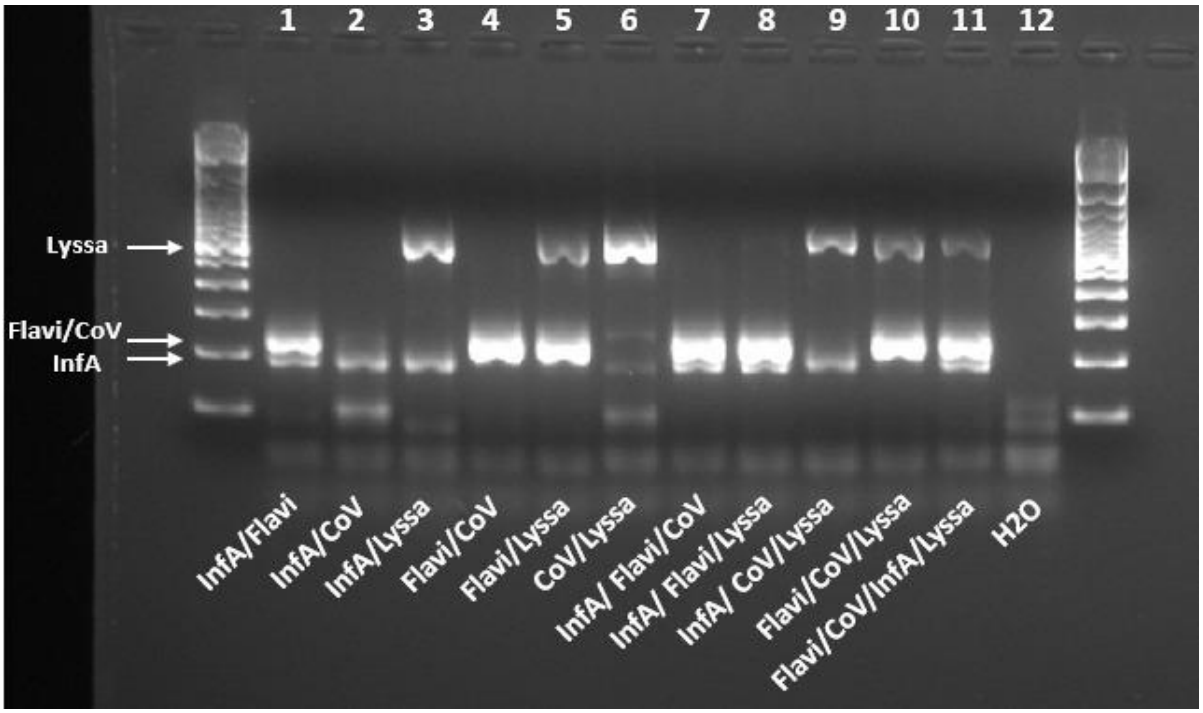


Figure 15: Gel image of PiiP primers used to detect multiple viral families in the same sample using a single mastermix and amplification cycle.

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

Continuation of the CANARIES Network

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

CANARIES continued in 2024, albeit virtually, with the meetings of the 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 2025.

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

COVID-19

Investigation of in vitro host-pathogen interaction between coronaviruses and bat cells.

The immortalized kidney cell line from Blyth's horseshoe bat, *Rhinolophus lepidus*, (Rhileki) was demonstrated to be susceptible and permissive for SARS-CoV-2. This is the first report indicating this *Rhinolophus* species as a potential host/reservoir for SARS-CoV-2-related viruses. The Rhileki cell lines were also used in isolation trials of SARS-CoV-2-like and swine acute diarrhea syndrome coronavirus (SADS) from Cambodian surveillance bat samples. In 2023, further work was extended to other bat cell lines (e.g., TB1 Lu purchased from ECACC) and primary bat cells. A paper was published in Microbiology Spectrum.

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

Axis 4: Rabies and other viruses

Diagnostic of Emerging Disease

Confirmation and Sequencing of MPXV genomes from Monkeypox outbreak in Phnom Penh, Cambodia (2023 – 2024)

As of February 17, 2025, Cambodia reported 20 local cases of human mpox, with the first case

identified on December 11, 2023, and the most recent case recorded on May 13, 2024, despite not having a historical record of mpox. Full genome sequencing of MPXV was successful for the 20 cases using AmpliSeq sequencing on Oxford Nanopore GridION technology. Phylogenetic analysis of the full genome revealed that the first case of the outbreak clustered most closely with a viral sequence (accession number: PQ368488.1) from Thailand (Figure 16, left) and was classified as Clade IIb, lineage C.1. Following this introduction event, the Cambodian outbreak genomes clustered into a single monophyletic group within the phylogenetic tree (Figure 16, left) and exhibited a high proportion of APOBEC3 mutations (Figure 16, right). Among the 19 mutations found exclusively within the outbreak genomes, 18 (94,7%) were APOBEC3-driven, while only one (5,3%) was linked to polymerase errors. These findings point to a single introduction event into Phnom Penh, followed by sustained human-to-human transmission, enabling local circulation of the C.1 lineage until at least May 13 2024. Phylodynamic analysis further supports the introduction of the C.1 lineage from Thailand to Cambodia on November 10, 2023, 31 days prior to the first detected mpox case and roughly nine and a half months after the ancestral split. The spatial backbone of the residual C.1 sequences, which drove the Cambodian outbreak, traced its path to Japan, and extended further into Southeast Asia, including Vietnam, Thailand and Cambodia. IPC Virology Unit continues to support the mpox response in Cambodia.

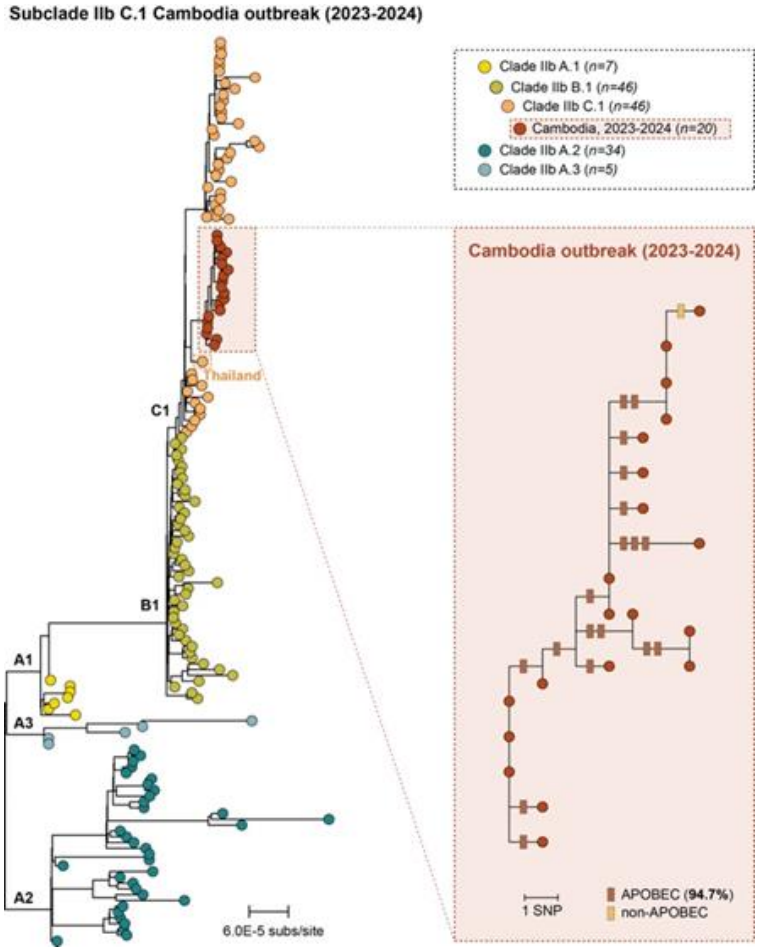


Figure 16: Maximum likelihood phylogeny of subclade IIb MPXV: Lineage C.1 introduction to Cambodia caused a 2023-2024 outbreak characterized by a dominance of APOBEC3-type mutations. In the left panel, the maximum likelihood phylogeny shows the placement of subclade IIb C.1 MPXV

(Orange) in Cambodia (Red). The viral genome most closely related to the Cambodian outbreak originates from a sample collected in Bangkok, Thailand, on July 4, 2023. The right panel shows an APOBEC3 analysis identifying 19 SNPs, of which 18 (94.7%) align with known APOBEC3-type deaminase editing signatures (TC-to-TT or its reverse complement, GA-to-AA). Other subclade IIb lineages are indicated as follows: A1 (light yellow), A2 (dark blue), A3 (light blue) and B1 (dark yellow).

Veterinary Virus Diagnosis

Molecular Diagnosis of EEHV in Elephants

Elephant Endotheliotropic Herpesvirus (EEHV), which causes a devastating hemorrhagic disease, is a significant threat to young Asian elephants, between one to eight years of age, with mortality rate up to 85%. The disease is characterized by sudden onset of illness, lethargy, edema, mild diarrhea, and fatal haemorrhages in all visceral organs, often leading to sudden death. In April 2024, the Virology Unit was contacted by the Elephant Valley Project coordinator from Mondulkiri province, Cambodia, for assistance with biological testing of samples from a 2-year-old female baby Asian elephant that had suddenly died on the same day of clinical symptom onset. Given the symptoms, which included lethargy and anasarca, EEHV was suspected as the primary cause of death. During a full necropsy, tissue samples were collected from heart, lung, spleen, intestine, tongue, skin, liver, and stomach. The diagnostic process started with SISPA metagenomics on heart and spleen specimens using ONT sequencing as the first approach, since the cause of death was unknown. The sequencing analysis indicated the presence of Elephantid betaherpesvirus 1 (EEHV-1), with 4,518 sequence reads in spleen and 81 reads in heart. With a depth coverage threshold at 5x (median: 13; IQR: 8 – 20), the breath coverage of EEHV-1 from the spleen sample was 91.1% of the virus genome. The phylogenetic analysis of the terminase gene showed that our EEHV-1 sequence was clustered with EEHV-1A strain from India, Europe and North America (Figure 17).

To confirm the results of metagenomic sequencing results, a qPCR targeted Major DNA-binding protein gene (U41) of EEHV1 was carried-out. All nine tissues collected from the baby elephant were tested positive, with Ct value ranging from 26 to 39.

On August 26, 2024, we received samples of two 4-year-old Asian elephants suspected of EEHV from Tamao Zoological Park and Wildlife Rescue Center (PTWRC). EEHV diagnosis was conducted using qPCR, and both elephants tested positive for EEHV-1. This early detection of EEHV was crucial for timely therapeutic decision-making and contributed to saving the elephant's life. Following these cases, we have implemented a longitudinal surveillance of EEHV in collaboration with Wildlife Alliance, monitoring the 8 (4 adults and 4 young elephants) elephants housed at PTWRC. As a result, EEHV-1 was detected in 3 young elephants, confirming active circulation of the virus among PTWRC elephants. IPC Virology Unit continues to contribute to ongoing EEHV surveillance efforts at PTWRC into 2025.

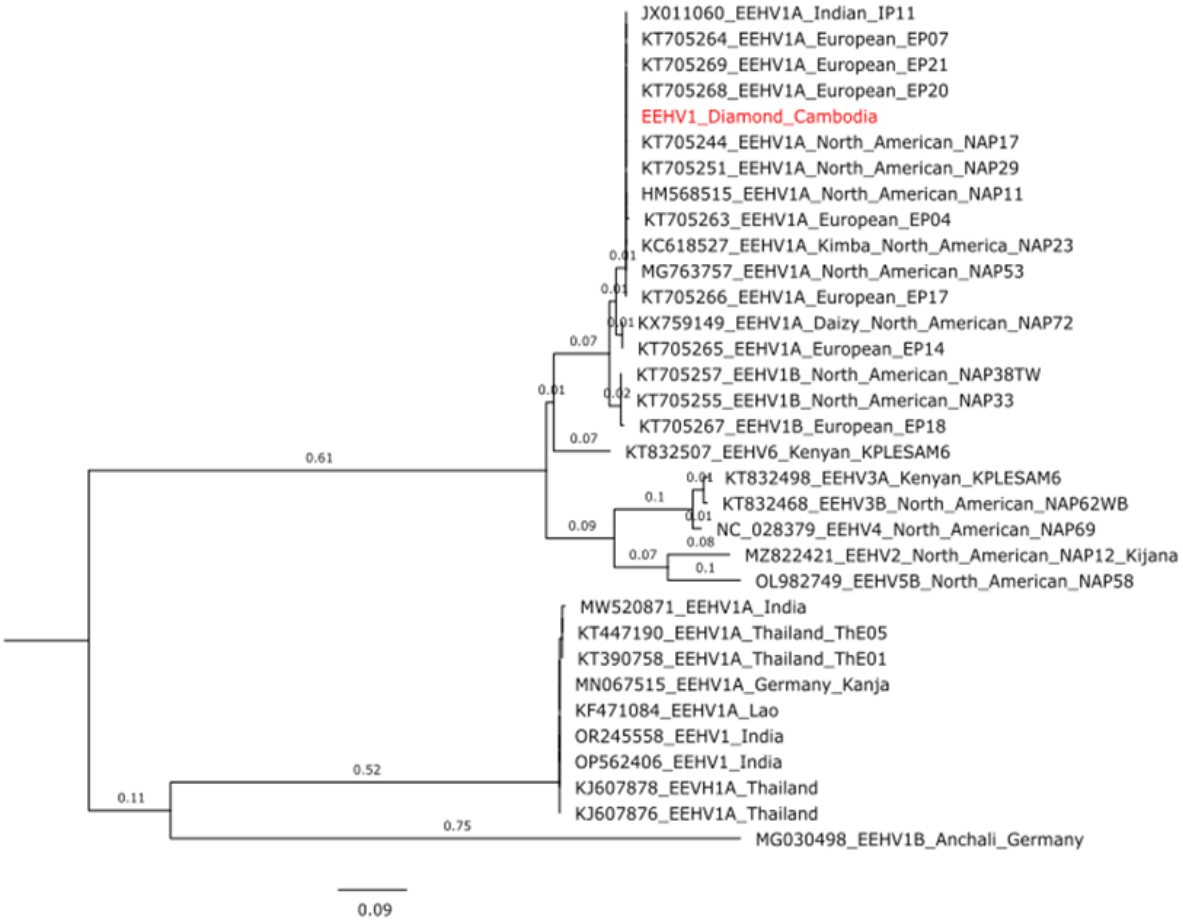


Figure 17: Phylogentic tree of EEHV terminase gene from Cambodian elephant detected in April 2024. Sequences were aligned and phylogenetic trees were constructed using MEGA v11.0.06 with the best-fit nucleotide substitution model (Tamura 3-parameter) chosen according to Bayesian Information Criterion (BIC). Trees were visualized and annotated using FigTree v.1.4.4. Cambodian EEHV-1 case is indicated in red.

Viral diagnostic in wildlife

In addition to the molecular diagnosis of EEHV in elephants, the Virology Unit provided support to PTWRC and Wildlife Alliance in testing of viral infection in wildlife including Leopard, bear, and pangolin presenting clinical symptoms (Table 6).

Table 6: Viral diagnosis in wildlife in 2024

Animal species	**Tested Number**	**Testing**	**Result**
Leopard	1	Lentivirus and Simain Foamy Virus	Negative
Douc Langur	1	Respiratory pathogens	See below
Sunda Pangolin	1	Influenza A, SARS-CoV2, and Coronaviruses	Negative
Bear	1	Metagenomics	No relevant pathogen detected

For Douc Langurs, testing of respiratory infections was conducted on 34 respiratory pathogens including SARS-CoV2 using Fast Track Diagnostic respiratory pathogens 33 multiplex assay (FTD33) and RT-qPCR. In December 2024, we received samples from a 1-year-old Douc Langur.
As a result, the animal tested positive for *Staphylococcus aureus, Streptococcus pneumoniae*, and *Klebsiella pneumoniae*. The testings are ongoing in 2025 as part animal health care efforts when introducing rescued animals into PTWRC.

Viral diagnosis in crocodile

Cambodia plans to export live crocodiles to the People's Republic of China in 2025. In compliance with Chinese custom requirements, all crocodiles must be free of West Nile virus, Japanese Encephalitis virus, and Crocodilian Herpesvirus infection. In 2024, the Virology Unit provided testing of these viruses to the Fisheries Administration of the Ministry of Agriculture, Forestry and Fisheries. Samples from two crocodiles raised in Siem Reap province were sent to IPC for testing, and one of them was tested positive for Crocodilian Herpesvirus 3.

Sequencing of ASFV genome from ASFV outbreak in Viet Nam

African swine fever virus (ASFV) poses a mjor threat to the swine industry. Understanding the genetic diversity of circulating strain is essential for disease surveillance, outbreak control, and the development of effective mitigation strategies. In 2024, the Virology Unit contributed to an effort in ASFV genome sequencing on samples collected in an outbreak event in Vietnam. In May 2024, We received blood and DNA samples isolated from three pigs tested positive for ASFV, with Ct values ranging from 19 to 29. We attempted full genome sequencing of ASFV for all samples using amplicon-based and metagenomics approaches including SISPA and shotgun sequencing.
As a result, only amplicon-based sequencing of one sample provided a significant number of sequence reads mapped to the ASFV genome. Consequently, the downstream analysis was focused on this sample. Using CZID bioinformatics pipeline (http://www.czid.org), most of sequence reads were mapped to ASFV sequence with NCBI accession #: ON963982, with 81% of genome coverage and median depth of 297x (IQR: 33x – 891x). The virus genotype was determined using full-length nucleotide sequence (1,941 bp) of the ASFV B646L (p72) region. The phylogenetic analysis indicated that our sample belongs to ASFV genotype II (Figure 17). In addition, the sequence analysis revealed that our sample exhibits complete deletions in the following genes: MGF 360-3L, MGF 360-12L, MGF 360-13L, MGF 360-14L, MGF 360 15R, and MGF 505-2R. Partial deletions were also observed in the following genes: MGF 360-4L, MGF 360-6L, MGF 360-18R, MGF 505-1R, MGF 505-3R, MGF 505-4R, MGF 505-5R, and MGF 505-10R. These findings suggested that our ASFV strain may belong to a vaccine-like variant with multiple gene deletions.
The Virology Unit continues to contribute to ongoing efforts to monitor the evolution of ASFV. A training in ASFV sequencing using Amplicon-based protocol is scheduled March 2025 in Hanoi, Vietnam.

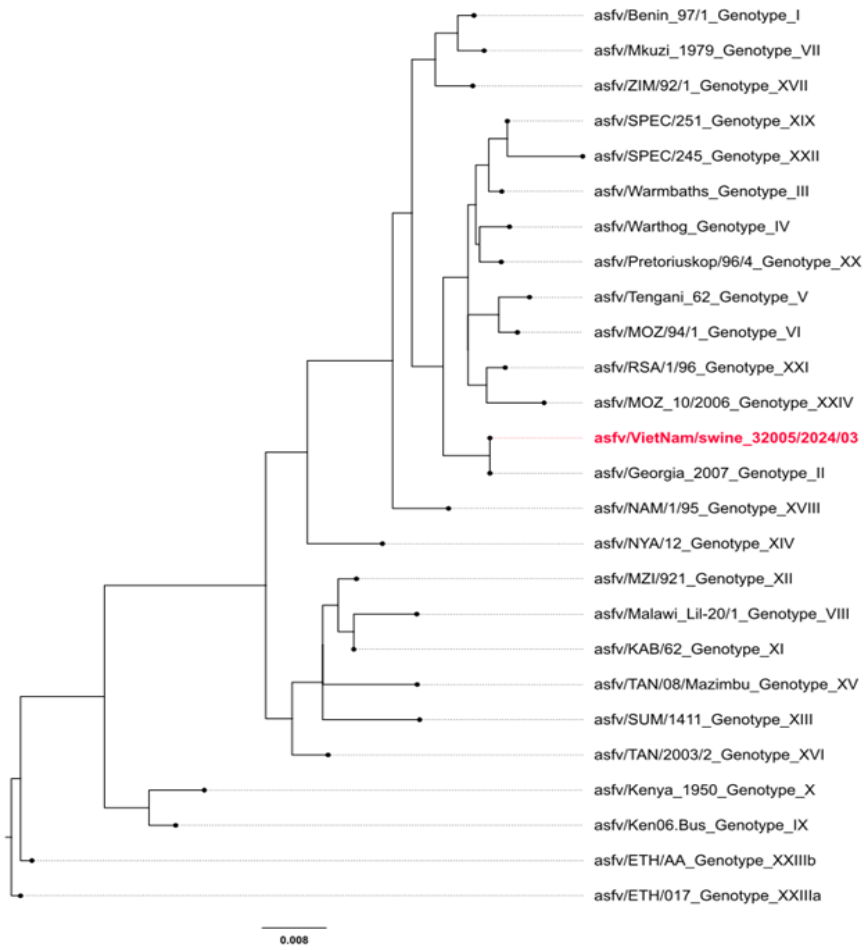


Figure 17: Maximum likelihood phylogenetic tree derived from full-length nucleotide sequences (1,941 bp) of the African swine fever virus p72 gene. *The sequences were aligned with ASFV representative genotype sequences described by Spinard et al., 2023 (PMID: 38005923) using MAFFT v.7.490 (PMID: 23329690) and phylogenetic tree were constructed using IQ-Tree v.2.0.3 (PMID: 32011700) with the best-fit nucleotide substitution model (TN+G) chosen according to Bayesian Information Criterion. Trees were visualized and annotated using FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and Inscape v.1.2 (http://www.inkscape.org). Our sample (ID#: 32005) is indicated in bold red.*

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

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

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

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

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

Starting from 2023, the Virology Unit's routine rabies diagnostic activities began to be documented in a FAO-supported veterinary public health Laboratory Information Management System (LIMS) known as SILAB. This system oversees all samples received for direct diagnosis by Direct Fluorescent Antibody test (DFAT), and for serological diagnosis by FAVNT, which is offered as a paid service. The implementation of SILAB facilitates faster result reporting to both customers and authorities. Following an assessment of the laboratory's needs for rabies diagnostic operations, two LIMS administrators were trained to manage rabies-related activities using SILAB. Additionally, four technicians were trained in sample and result entry. The adoption of the SILAB LIMS enhances sample tracking, testing, and reporting, and improves the overall management of the Virology Unit's diagnostic capabilities. The SILAB LIMS system has been in regular usage and expanded in 2024.

Research Project Name	SILAB-IPC
Funding	Food and Agriculture Organization of the United Nations (FAO)
Project duration	2023 – 2024
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson, Heidi Auerswald); Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM, Ecole DEL NEGRO)

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

Cervical cancers remain a public health concern in most countries. It is caused by sexually acquired infection with human papillomavirus (HPV), notably genotypes 16 and 18. Several studies have suggested associations between oncogenic potential of HPV and viral genetic variabilities including E6 and E7 coding regions that play important roles in cervical carcinogenesis. The main objective of the study is to describe and characterize HPV diversity circulating in HIV-infected women in Cambodia using the NGS approach. Secondarily, we will assess the associations between variants in HPV E6 and E7 coding genes and cervical intraepithelial neoplasia (CIN) status.

A total of 60 women co-infected with HIV and HPV were included in the study. Rolling-circle amplification metagenomics approach and Illumina MiSeq technology have been used to obtain HPV whole genome. The sequencing activities were completed for all included participants (20 in 2022 and 40 in 2023). In 2024, our activities focused on sequence analysis. The sequencing data analysis pipeline includes a quality check of the sequence read, followed by read classification, identification of HPV predominant strain, depth and breadth coverage assessment, and variant analysis.

After the classification step, 32 out of 60 samples had more than 10 HPV sequence reads. The high-risk HPV16 and HPV18 were detected in 6/32 and 4/32, respectively. The 22 remaining samples were infected with other genotypes including HPV33, HPV39, HPV58, and HPV70. Most participants were infected with multiple HPV genotypes. Due to the limited number of samples per genotype, further analysis focused on the 10 participants infected with HPV16 or HPV18. Among the 6 HPV16-positive participants, five were co-infected with other high-risk genotypes including HPV30, HPV33, HPV35, and HPV91. All of HPV18 participants were mostly co-infected with a low-risk HPV97 (Table 6). The analysis of virus variants revealed that the E6 region exhibits variability within HPV16, whereas no variants were detected for HPV18. Among the 4 individuals infected with HPV16 and diagnosed with CIN2+, three harbored at least one virus variant at one position in E6 and E7 regions. One CIN2+ individual had variants at 8 positions in E6 and 3 positions in E7 regions. Among individuals having CIN grade below 2, one had a variant at a single position of E6 and 2 positions of E7, while no variants were observed in another. Further study is needed to investigate the biological significance of these variants.

Table 7: Sequence coverage of HPV16 and HPV18 positive samples

Sample ID	CIN	Top Strain	Next Highest Strain	Genome Depth Coverage	Genome Breadth Coverage	E6 Coverage Depth	E7 Coverage Depth
64	CIN2+	HPV16	HPV33	27,000 X	100%	8,000 X	3,000 X
149	CIN2+	HPV16	HPV35	9,000 X	100%	2,000 X	979 X
298	CIN2+	HPV16	HPV91	24,000 X	100%	6,000 X	2,000 X
306	CIN2+	HPV16	HPV35	24,000 X	100%	7,000 X	7,000 X
152	CIN2-	HPV16		238,000 X	100%	57 X	20 X
186	CIN2-	HPV16	HPV70/HPV 30	3,000 X	100%	724 X	313 X
213	CIN2+	HPV18	HPV97	13,500 X	98%	497 X	2,600 X
229	CIN2+	HPV18	HPV97	2,400 X	97%	309 X	2,000 X
75	CIN2-	HPV18	HPV97	13,500 X	100%	2,000 X	11,800 X
237	CIN2-	HPV18	HPV97	4,700 X	100%	741 X	3,900 X

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

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

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

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

3.4.3 Scientific Trainings, Workshop and Symposia

Scientific Trainings

- Erik Karlsson, Jurre Siegers, Giorgio Gonnella, Indonesia, August 5 – August 9 2024, **Follow-Up Bioinformatics Training Workshop (NGS-Nanopore Technology)** for the Directorate General of Livestock and Animal Health Services & Partners
- Erik Karlsson, Jurre Siegers, Sokhoun Yann, Vietnam, October 14 – October 17 2024, **Genomic Sequencing of Avian Influenza**
- Jurre Siegers, Sokhoun Yann, Cambodia, May 27 – May 30 2024, **Influenza sequencing on ONT Techinque and bioinformatics using IRMA tool** for Thai NIC
- Anna S. Fomsgaard, Austria, November 18, 2024 - November 29, 2024, **Next Generation Sequencing and Nanopore Sequencing Applications for the Detection and Characterization of Pathogens** for the VETLAB Network at the joint FAO/IAEA laboratory

Workshops

Consultative Workshop: Enhancing and applying effective communication & Reporting strategies, risk mitigation measures, & prevention using Environmental Surveillance at high-risk Live Animal Markets and other interfaces

On 15, 16 and 17 May 2024, the Consultative Workshop: Enhancing and Applying Effective Communication and Reporting Strategies, Risk Mitigation Measures, and Prevention Using Environmental Surveillance at High-Risk Live Animal Markets and other Interfaces was held in Phnom

Penh at Phnom Penh Hotel. The workshop was organized by Institut Pasteur du Cambodge (IPC) and Labyrinth Global Health.

A total of 45 attended the workshop, including representatives from Ministry of Agriculture, Forestry and Fisheries (MAFF), Ministry of Health (MoH), Ministry of Environment (MoE), the Asia Pathogen Genomics Initiative (Asia-PGI), the EcoHealth Alliance (EHA), the Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO), United States Agency for International Development (USAID), and Duke-NUS. The workshop used an interactive methodology and a structured approach with user-friendly materials, simulation exercises, and facilitation tools. All participants received a Concept Note, which comprised all necessary information, such as the objectives of the workshop, instructions for working group exercises, expected outcomes, etc. Sessions were structured in a step-by-step process as detailed in the following pages of this report.

Dr. Karlsson provided the closing remarks with a comprehensive recap of all the discussions held throughout the various sessions of the workshop. Based on the preferences expressed by the participants, a prototype dashboard was developed. This dashboard was designed to significantly improve the government's capability to effectively communicate metagenomic data and implement informed actions (Figure 13). It was demonstrated to facilitate quick and accurate decision-making, promoting a more coordinated and effective response among different ministries and stakeholders. Dr. Karlsson extended his deepest gratitude for everyone's active participation and continued commitment to enhancing ES in LBMs.

Organizers	Frida Sparaciari, Malen Chan, Karen Saylors, Sofia Perez, Erik Karlsson
Funding	The Gates Foundation
Dates	May 15, 2024 - May 17, 2024
Collaboration	Institut Pasteur du Cambodge : Virology Unit Labyrinth Global Health

Symposia

The Pasteur Network Asia-Pacific Avian Influenza Symposium and Workshop 2024

The Pasteur Network Asia-Pacific Avian Influenza Symposium and Workshop, held at the Hyatt Regency Hotel in Phnom Penh, Cambodia, marked a historic milestone in regional collaboration. The event was funded by the Pasteur Network and Institut Pasteur du Cambodge. For the first time, all nine Asia-Pacific Pasteur Network institutes—Cambodia, Hong Kong, Iran, Korea, Laos, New Caledonia, and Vietnam (Hanoi, Ho Chi Minh City, and Nha Trang)—were represented at a single event held in the region. With up to 74 attendees daily, including development partners (World Health Organization and World Organization for Animal Health), research partners (Duke-NUS, University of Georgia), private partners (HumanLink, Biotia), and officials from the Cambodian Ministry of Health and Ministry of Agriculture, Forestry, and Fisheries, the symposium underscored the critical importance of regional cooperation in addressing avian influenza virus (AIV).

The symposium concluded with a closing session led by Dr. Erik Karlsson, reflecting on the event's achievements and outlining next steps. The roadmap, a major outcome of the workshop, provides a comprehensive strategy for advancing influenza research and enhancing preparedness across the Asia-Pacific Pasteur Network.

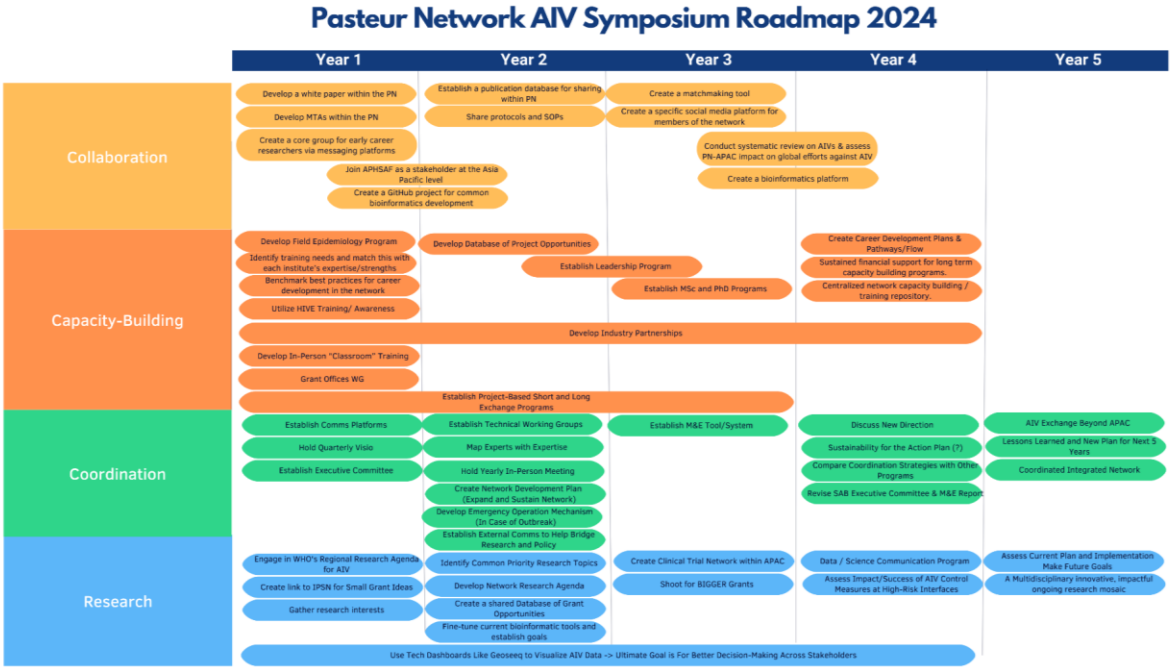


Figure 18: Visualization of the 5-Year Roadmap following Prioritization Workshop Session based on Four Pillars 'Collaboration', 'Capacity-Building', 'Coordination' and 'Research'

Organizers	Erik Karlsson, Vijaykrishna Dhanasekaran, Khaoula Abidi, Sofia Perez, Alexandre Blaise, Marea Pheap
Funding	Pasteur Network, Institut Pasteur du Cambodge
Dates	December 10, 2024 - December 12, 2024
Collaboration	Institut Pasteur du Cambodge: Virology Unit HKU-Pasteur Pasteur Network

3.4.4 Teaching and Students

PhD students

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

Master students

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

Bachelor students

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

Teaching

- HORM Sreyviseth, course "Infectious Disease agents" (23h) for Foundation Year – Infectious Disease at University of Health Sciences in Phnom Penh
- HORM Sreyviseth, course "Respiratory Infection" (6h) for 5th Year Pharmacy – Clinical Biology at University of Health Sciences in Phnom Penh
- HORM Sreyviseth, integrated course (4h) for 5th Year Pharmacy at University of Health Sciences in Phnom Penh
- HORM Sreyviseth, "MMR" (3h) for Master's Students - Microbiology for Medical Biology Sciences at University of Health Sciences in Phnom Penh
- MEY Channa, course "Active and Passive Immunotherapies" (2h) for 3rd Year Medicine - Medical Immunology at University of Health Sciences in Phnom Penh
- MEY Channa, course "Immunodiagnostic Laboratory" (17h) for Master's Students - Medical Immunology at University of Health Sciences in Phnom Penh
- Ou Tey Putita, course "Structure-Taxonomy-Multiplication of virus, Coronaviridae (SARS-CoV, Mers-CoV and other), Interpretation of arbovirus serology assay, Serology (ELISA), PCR (real time RT-PCR) and sequencing" (2h) Master's Students - Microbiology for Medical Biology Sciences at University of Health Sciences in Phnom Penh

Received Training

- **Y Phalla** & **PHOU Yisuong** participated in the Luciferase Immunoprecipitation System (LIPS) training in Laos from 27/10/2024 to 07/11/2024. This 12-day session covered the use of LIPS for detecting antibodies and protein interactions, including assay principles and troubleshooting. It was hosted by Institut Pasteur du Laos, and other attendees were from IPL. The training was held in Laos.
- **KEN Sreymom** & **THET Sopheak** took part in a workshop on developing SOPs for sample transportation and strengthening laboratory capacity in Cambodia from 26/08/2024 to 29/08/2024. The 3-day training aimed to improve surveillance and outbreak management through effective sample transportation. The event was organized by DHS, MOH, and FHI360, with other attendees from MOH, FHI360, CDC, GIZ, WHO, and laboratories from hospitals and provincial areas. The workshop took place in Cambodia.
- **BUN Kimrong** participated in a 11-day training on sero-neutralization of arboviruses at Hokkaido University International Institute for Zoonosis Control in Japan from 07/10/2024 to 18/10/2024. The training focused on sero-neutralization tests and analysis of arthropod-borne viruses. It was organized by PICREID, and other attendees were from Hokkaido University.
- **PUM Leakhena** attended the GISAID-ASEAN Bioinformatics Training for Viral Pathogens Workshop in Malaysia from 03/05/2024 to 03/07/2024. This 3-day workshop offered bioinformatics training for viral pathogens. It was organized by NIPH and GISAID, with participants from NIPH, and took place in Malaysia.
- **YANN Sokhoun & RITH Chenthearath** completed the WHO Bioinformatic Workshop on strengthening genomic surveillance of influenza, SARS-CoV-2, and RSV in Australia from 28/09/2024 to 03/10/2024. The 5-day training enhanced skills in genomic surveillance and data interpretation using bioinformatics tools. It was organized by NIPH and WHO, with participants from NIPH. The workshop took place in Australia.
- **PUM Leakhena & NOUN Sithun** participated in a training on genomic sequencing for zoonotic pathogen spill-over prevention at Mahidol University in Thailand from 26/05/2024 to 01/06/2024. The 6-day training covered sample collection and genomic sequencing methods. It was organized by TDRC and Mahidol University, with attendees from Institut Pasteur du Laos and Mahidol University. The training was held in Thailand.
- **KHUN Limmey** attended a Bioinformatics training on viral genomics and bioinformatics in Vietnam from 05/12/2024 to 05/17/2024. The 6-day hands-on program covered metagenomic experiments and NGS data analysis. The training was organized by Wellcome Connecting Science and Oxford University Clinical Research Unit, with other participants from across Asia, and was held in Vietnam.
- **KOL Sonita** participated in a workshop on Multi-Pathogen Genomics in Wastewater Surveillance in Singapore from 07/10/2024 to 11/10/2024. This 5-day workshop covered wastewater sampling, genome enrichment, and bioinformatics techniques. It was organized by the ASIA Pathogen Genomics Initiative and held in Singapore.
- **Julia GUILLEBAUD** participated in a 3-day workshop looking at Bayesian latent class models for prevalence estimation and diagnostic test evaluation. The event was held in Australia from 18/11/2024 to 20/11/2024. The session used veterinary examples and focused on R and JAGS. The training was organized under the 17th International Symposium on Veterinary Epidemiology and Economics (ISVEE17), with other participants from various scientific fields.

- **SOUR Kimhoung** & **KHUN Limmey** attended a training for researchers and technicians on innovative techniques for diagnosing zoonotic infections in Laos from 18/09/2024 to 04/10/2024. This 18-day training covered pathogen discovery through bioinformatics, focusing on viral proteins, homology modeling, and structural analysis. It was organized by IPL and IP-Paris, with attendees from IPL. The training was held in Laos.
- **YANN Sokhoun** attended an Accelerated NGS Bioinformatics training in Indonesia from 18/04/2024 to 21/04/2024.
- **Frida Sparaciari** attended a 5-day DTHM Cohort Doctoral Studies Program in Australia from 05/02/2024 to 09/02/2024. The program provided support and community-building for HDR students. It was organized by JCU and held in Australia.
- **Frida SPARACIARI** participated in a Basic Statistics with R and RStudio workshop in Australia from 09/09/2024 to 11/11/2024. The event provided fundamental statistical skills using R software. It was organized by JCU.
- **Frida SPARACIARI** took part in a 3-day workshop on Artificial Intelligence and its applications in food systems in Italy/online from 23/09/2024 to 25/09/2024. The workshop was organized by EFSA and held both online and in Italy.
- **Frida SPARACIARI** attended a 2-day Thematic Analysis Research Methods Masterclass in Australia from 14/10/2024 to 15/10/2024. The masterclass focused on qualitative research methods, including data collection and analysis. The training was organized by JCU and held in Australia.
- **OU Tey Putita** attended a session on Improving Basic Knowledge of Biotechnology in France from 15/03/2024 to 30/11/2024.
- **OU Tey Putita** attended the Asia Pacific Symposium on Tropical Diseases in South Korea from 20/02/2024 to 22/02/2024. The 5-day event focused on the latest molecular tools for infectious disease research. The training took place in South Korea.
- **OU Tey Putita** participated in a 1-day Personal Management training on assertiveness and team management in France on 08/01/2024. The session focused on enhancing personal impact in team settings. It was organized by Montpellier University and held in France.
- **OU Tey Putita** took part in a training on preparing for conferences in France from 25/03/2024 to 29/03/2024. This 5-day training focused on improving oral and poster communication skills. It was organized by Montpellier University and held in France.
- **OU Tey Putita** attended 'IBEID - Adapting to Change' in France, organized by Institut Pasteur du Paris.
- **Leangyi HENG**, **Songha TOK**, and **Sopheak THET** attended a Bio-Risk Management Workshop in Cambodia from 23/07/2024 to 26/07/2024. This 3-day workshop raised awareness about biosecurity, risk assessment methodologies, and countermeasures to mitigate risks. The training was organized by Centres of Excellence (EU Initiative) and the UK Health Security Agency, and took place in Cambodia.
- **BUN Kimrong**, **CHANTHA Soursdey**, **CHEA Kimlay**, **HENG Leangyi**, **IN Saraden**, **KEN Sreymom**, **Limmey KHUN**, **Y Phalla**, **YUN Chanvannak**, and **THET Sopheak** attended the Quality Assurance and Good Laboratory Practice in Analytical Testing training virtually from 22/11/2023 to 01/03/2024. The 3-month training covered various QA and GLP topics, including validation of methods, traceability, and proficiency testing. It was organized by UNIDO and the Department of Fisheries Post-Harvest Technologies (FiA), and was attended by other team members from IPC's various Units.

- **SAULIM Sereyrith, KHUN Limmey, PHUM Leakhena, Sophoannadedh RATH, Sonita KOL, Teyputita OU, Anna Signe FOMSGAARD and Kathrina Mae BIENES** attended a Command Line Basic Tutorial held by the Bioinformatics and Artificial Intelligence Applications Unit at Institut Pasteur du Cambodge.
- **BUN Kimrong, CHANTHA Soursdey, CHEA Kimlay, HENG Leangyi, IN Saraden, KEN Sreymom, PHOUT Yisuong, YUN Chanvannak, THET Sopheak** attended a virtual session on ISO 17025:2017 Awareness over a 1-month period organized by UNIDO and Department of Fisheries Post-harvest Technologies (FiA).
- **HENG Leangyi** attended the ISO 15189:2022 Certified Auditor Training virtually from 01/08/2024 to 01/09/2024. This 2-day session focused on becoming a certified auditor for ISO 15189:2022 and evaluating management systems. It was organized by Exemplar Global and Punyam Academy.
- **HENG Leangyi** attended the ISO 15189:2022 Awareness Training virtually on 01/08/2024. This 1-day session raised awareness about ISO 15189:2022 in medical laboratories. It was organized by Punyam Academy.
- **HENG Leangyi** completed the ISO/IEC 17025:2017 Certified Auditor Training virtually on 14/01/2024 and 15/01/2024. The 2-day training focused on becoming a certified auditor for ISO/IEC 17025:2017 and evaluating management systems. It was organized by Exemplar Global and Punyam Academy.
- **HENG Leangyi** participated in a Fire Prevention and Firefighting Training in Cambodia from 02/02/2024 to 03/02/2024. The 1.5-day training covered fire prevention methods and firefighting techniques. It was organized by IPC and held in Cambodia.
- **HENG Leangyi** completed a Cardiopulmonary Resuscitation (CPR) Training on 29/08/2024 in Cambodia. This 3-hour training focused on using automatic CPR machines and emergency response. It was organized by IPC and MC&Co Prevention Co., Ltd., and held in Cambodia.
- **HENG Leangyi** attended the ISO 35001:2019 Certified Auditor Training virtually on 19/12/2024 and 20/12/2024. The 2-day training focused on ISO 35001:2019 certification and management system evaluation. It was organized by Exemplar Global and Punyam Academy.
- **HENG Leangyi** participated in the ISO 35001:2019 Biorisk Management Awareness Training on 18/12/2024. The 1-day session focused on raising awareness of ISO 35001:2019 in biorisk management. It was organized by Exemplar Global and Punyam Academy.

3.4.5 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. The surveillance data between 2000 and 2024 reveals that there were four big dengue outbreaks observed in 2007 by DENV-3, in 2012 by DENV-1, in 2019 by DENV-1 and in 2023 by DENV-2. DENV-4 circulates at background level. The genomic data showed that there was constant replacement of serotypes/genotypes/lineages and these replacements were often associated with big dengue outbreaks.

In 2024, the Virology Unit received 795 samples from suspected dengue patients admitted to the sentinel hospitals and 528 were confirmed positive by RT-PCR (66.4%). DENV-2 (n=436; 54.8%) was the major serotype detected followed by DENV-4 (n=65; 8.2%), DENV-1 (n=27; 3.4%) and DENV-3 (0.0%).

Besides, DENV, Zika virus (ZIKV) is reported to be endemic in Cambodia but in low prevalence. Chikungunya virus (CHIKV) was introduced in Cambodia in 2011 and in 2020. The virus was not able to become endemic and disappeared respectively in 2013 and 2022.

Cambodian National Influenza Center

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

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

Human seasonal influenza

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

The current global COVID-19 pandemic has significantly altered both the surveillance and landscape of respiratory diseases worldwide. Indeed, the introduction of control measures in early 2020 to reduce the transmission and disease burden of SARS-CoV-2 infection has shown a remarkable reduction in the infection rates of many respiratory diseases despite continued, or even increased, testing for influenza in some countries.

Once international border restrictions were eased on 11 June 2020, Cambodia experienced an outbreak of influenza A(H3N2), that circulated in several provinces from July through to November 2020 including clustered detections in closed/semi-closed systems (prisons/pagodas), and also spreading in the general community. The prototypical Cambodian A(H3N2) strain from this period, A/Cambodia/e0826360/2020, was selected as the recommended composition for use in the 2021–2022 northern hemisphere influenza vaccine in February 2021. This virus was identified and first isolated at IPC, and Cambodia's inclusion in the vaccine's development represents a first for the country. A paper detailing the initial A(H3N2) outbreaks in mid- to late-2020 was published, and a second one which details the genetic and antigenic findings from the entire outbreak was also published in 2021.

Following the 2020 outbreak, IPC began to follow recommendations from the WHO's guidelines to maintain influenza surveillance, especially with the exceptionally low numbers of influenza infections

detected worldwide in 2020 and 2021. Out of 4421 samples from symptomatic patients presenting an influenza-like illness or SARI during COVID-19 screenings in 2021, no human seasonal influenza was detected in Cambodia. In 2022, similar to the rest of the world, influenza began to rise again in Cambodia with an initial wave of A(H3N2) followed by Influenza B. In 2024, Cambodia experienced two waves of influenza, the first dominated by B/Victoria and the second dominated by a mixture of A/H1N1pdm and A/H3N2 (Figure 19)

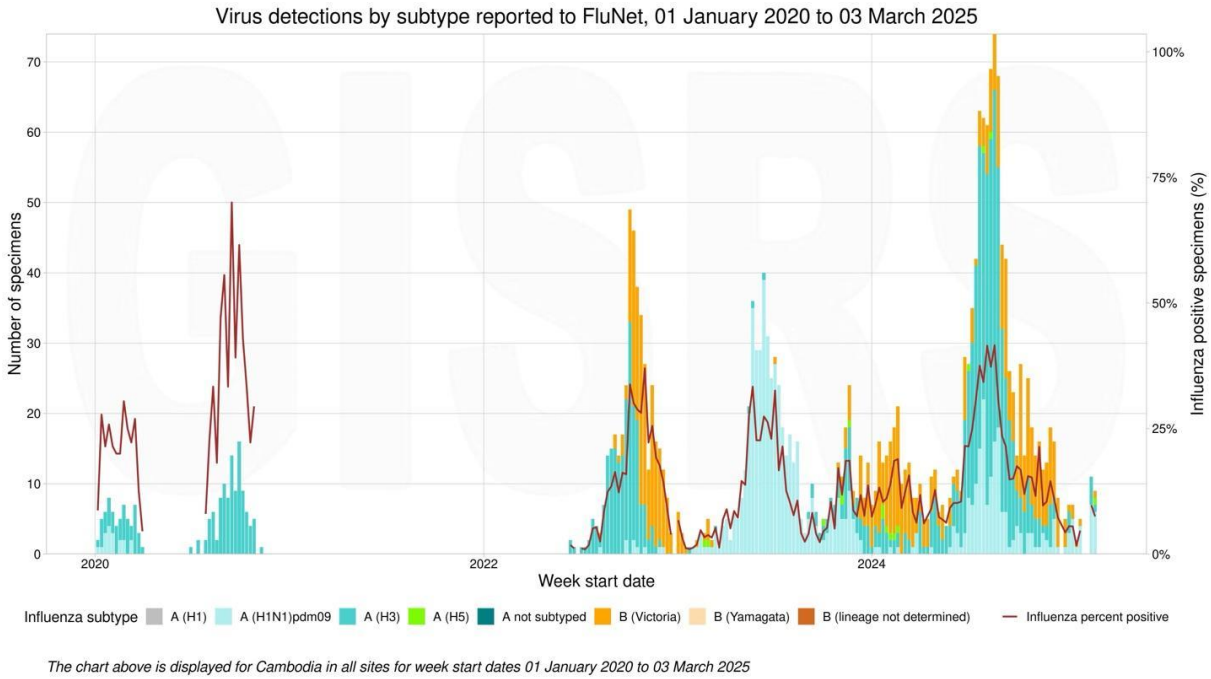


Figure 19: Number of influenza cases detected in sentinel and outbreak samples 2020-2024. No influenza cases were detected in Cambodia in 2021. Influenza returned to Cambodia in 2022. Data source: FluNet (https://www.who.int/tools/flunet)

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 2024 with only a limited number of samples tested. Sequencing, analysis, and publication are underway to describe these viruses in Cambodian children expected mid- to late 2025.

Detection and response to A/H5N1 infections in humans in 2023 – 2024.

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

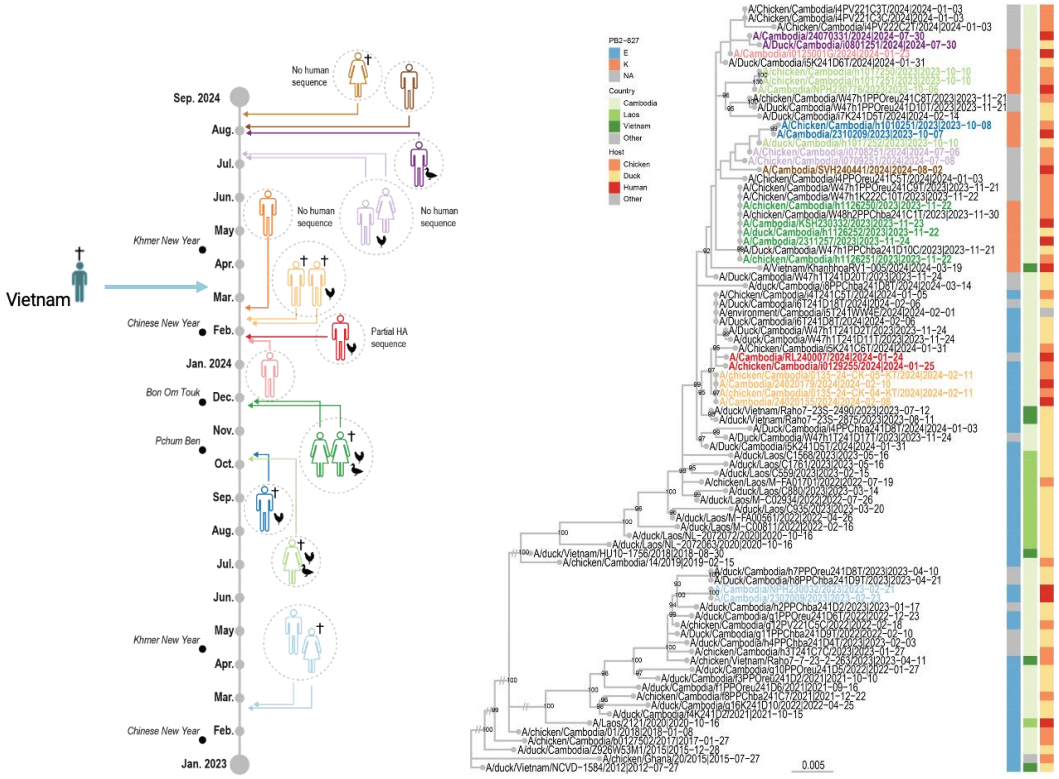


Figure 20: Phylogenetic subtree of A/H5Nx 2.3.2.1c hemagglutinin (HA) gene for typing of human A/H5N1 cases detected in Cambodia in 2023-2025. Sequences were aligned using MAFFT v.7.490 (PMID: 23329690), trimmed using trimAL (PMID: 19505945) and phylogenetic trees were constructed using IQ-TREE v.2.0.3 (PMID: 32011700) with the best-fit nucleotide substitution model (GTR+F+I+G4 chosen according to Bayesian

Information Criterion (BIC)). Trees were visualized and annotated using FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and Adobe Illustrator 27.3. Human A/H5N1 cases are highlighted in bold red.

WHO Global COVID-19 Reference Laboratory

Novel coronavirus disease 2019 (COVID-19)

Following the detection of a cluster of cases of pneumonia of unknown etiology in Wuhan, China in December, 2019, on 27 January, IPC confirmed the first COVID-19 case (a traveller from Wuhan) in Cambodia. In April 2020, the work done at IPC in response to the global COVID outbreak was recognized by designating the Virology Unit as a WHO COVID-19 global reference centre. In addition, IPC continues working closely with the Cambodian CDC, which is the coordinating entity designated for notification of suspected cases, and the COVID-19 sampling system as a whole. While testing significantly decreased since 2022, by 31 December 2024, IPC had tested over 945,040 samples (~500 samples in 2024) for SARS-CoV-2 by RT-PCR and identified (or confirmed when first identified by NPHL at NIPH or one of the regional laboratories) tens of thousands of positive cases as part of surveillance and response, including the ongoing community transmission events.

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

Even before COVID-19 was declared a public health emergency of international concern (PHEIC), IPC was designated by the government of Cambodia as a reference to validate and verify all novel and incoming assays for SARS-CoV-2 before their use in the Kingdom. In 2021, IPC validated five of these kits, and this data is frequently shared with the global network. In addition, technology transfer has been achieved with NIPH, Sihanoukville, and Ket Mealea laboratories as beneficiaries to help ensure that testing for all VoCs is widely available in the country. A publication detailing this work is expected by mid-2024.

Based on the validation/verification and usability of the VoC RT-PCR kits tested, IPC decided to employ the KogeneBiotech PowerChek™ SARS-CoV-2 S-gene mutation kit in daily routine genomic surveillance. IPC can test 92 samples/day (644/week) in routine testing of new cases in Cambodia. These samples are chosen based on the availability of what was tested the previous day and selected from as many provinces and sample types as possible. In addition, IPC does daily VoC testing for the Siem Reap and Battambang laboratories, and confirmatory testing for NIPH and KTML laboratories as they also employ these kits in daily testing. Between January and December 2024, IPC had tested over 70 samples for VoC by RT-PCR to monitor Variants of Concern (VoC) circulating in and entering Cambodia (Figure 20).

SARS-CoV-2 Sequencing

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

Between January 2020 and the end of December 31st 2024, IPC was able to sequence 3,405 samples (2.44 % of the total reported cases at that time) and submitted results to GISAID (Figure 21). Further work continues to monitor mutation rates in vaccinated versus unvaccinated individuals, transmission

dynamics, and the phylogeography of the viral spread. Two manuscripts have been written regarding SARS-CoV-2 sequencing in Cambodia. The first detailing the results from 2020 has been published in early 2023, the second looking at details from 2021 was published at *Communications Medicine* in 2024 (see below).

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 traveler 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 23). 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.

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

Tracking the emergence, introduction and spread of SARS-CoV-2 variants of concern are essential for informing public health strategies. In 2021, Cambodia faced significant challenges from two major epidemic waves caused by Alpha and Delta variants. Complete SARS-CoV-2 genomes from 1,163 COVID-19 patients in Cambodia from February–September 2021 were underwent evolutionary analyses to explore lineage diversification, and to infer virus population dynamics and transmission. The correlation between epidemic occurrence and public health control strategies were examined. Phylogeographic reconstruction was conducted to infer the spatiotemporal processes of Alpha and Delta variants. During the first wave, Cambodian Alpha variant diversified and rapidly expanded reaching an effective reproductive number exceeding 3.0 in March 2021. Subsequently, it acquired a spike E484K mutation, coinciding with the country's mass vaccination campaigns in April 2021. The Delta variant, with relatively stable growth, quickly displaced the Alpha variant, possibly due to key amino acid mutations associated with increased infectivity and transmissibility. Our findings suggest that the Alpha variant entered Cambodia through the capital city Phnom Penh, before spreading nationwide. In contrast, the Delta variant was likely introduced via northern border provinces before disseminating to Phnom Penh and other provinces (Figure 24). Despite nationwide control measures and high vaccine coverage, the Delta variant rapidly replaced the Alpha variant, becoming predominant in Cambodia by August 2021. Genomic surveillance combined with public health strategies was vital for effectively tracking and responding to the emergence and dissemination of emerging variants.

Viral Isolation Titration

Having a BSL-3 level facility and experience in isolating numerous types of viruses, IPC was quickly able establish viral isolation and tittering (both TCID50 and PFU) for COVID-19 in Cambodia. At the current time, 129 viral isolates (Wuhan-like, Alpha, and Omicron) are available from patients identified in Cambodia. We have as yet been unsuccessful in isolating the Delta VoC. This strain biobank is vital, not only for our continued validation and technical improvement work, but also for establishing serological assays for sero-epidemiological surveys and contact-tracing efforts. Further isolation attempts are made on all possibly viable samples available.

Diagnostic for rabies infection in animals

NAHPRI Training for Enhanced Rabies Diagnostic Capacity

To enhance the post-mortem rabies diagnostic capabilities at the National Animal Health and Production Research Institute (NAHPRI), a collaborative initiative was launched in partnership with the GIZ-funded support to the Royal Government of Cambodia. This is part of the GIZ global programme for Pandemic Prevention and Response, One Health. The Virology Unit lends its technical expertise in rabies direct diagnostic through a twinning approach with NAHPRI. The focus is on diagnosing rabies virus in animal brain samples using the direct fluorescence antibody test (DFAT). This training was aimed at building their capacity in conducting DFAT for rabies virus. Throughout the project, quality assurance measures such as confirmatory testing and sample sharing upon request will be implemented to ensure the reliability and accuracy of the testing conducted at NAHPRI.

2024 Rabies Surveillance: Post-Mortem Diagnostic Outcomes at IPC Rabies Prevention Centers

The rabies surveillance activities conducted in 2024 encompassed free post-mortem diagnostics of animals involved in bite incidents, which were brought to one of the three IPC rabies prevention centers. This initiative was crucial in tracking and managing the incidence of rabies across different regions. In 2024, a total of 187 animals were tested for rabies (168 dogs, 17 cats, 1 monkey, 1 squirrel). Out of these, 64.7% were found to be positive for rabies virus (119 dogs and 2 cats tested positive). 70.8% of all dog samples (119/168) were positive for rabies, 11.7% of all cat samples (2/17) were positive for rabies.

The majority of these samples came from Prey Veng, indicating a possible higher incidence of rabies in this region compared to others.

Province	Samples Tested	Positive	Prevalence
PP	22	5	22.7%
Kandal	4	2	50.0%
Prey Veng	48	32	66.7%
Kampong Speu	12	6	50.0%
Takeo	16	11	68.8%
Kampong Cham	13	10	76.9%

Kampot	19	17	89.5%
Svay Rieng	17	13	76.5%
Kampong Chhang	8	5	62.5%
Pursat	1	1	100.0%
Battambang	7	5	71.4%
Kampong Thom	10	7	70.0%
Preah Vihear	2	2	100.0%
Kratie	1	1	100.0%
Tboung khmom	5	4	80.0%
Stoeng Treng	1	0	0.0%
Kep	1	0	0.0%
Total	**187**	**121**	**64.7%**

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

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

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

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

3.4.6 Outlook for upcoming 3 – 5 years

Research Projects

RACSMEI

We aim to develop an integrated cost-effective multidisciplinary research framework to enhance Precision Public Health in a LMIC country, by studying the major priority pathogens in a One Health perspective. It will generate a considerable amount of knowledge and facilitate the development of targeted strategies for public and animal health. Our multidisciplinary consortium aims to conduct a nationally-representative cross-sectional survey on zoonotic, endemic diseases using a multiplex detection approach at the human-animal-human-environmental interface, across 10,000 randomly selected individuals, domestic/peri-domestic animals, vector species, and the environment. The program will benefit from the latest innovations in the fields of multiplex serology, environmental sampling, metagenomics and modelling.

We will address several key research questions related to epidemiology, ecology, and transmission of infectious diseases in Cambodia, critical for generating and implementing effective public health interventions. These questions encompass understanding the burden and spatial transmission risks within human and animal populations, as well as identifying the key determinants of transmission dynamics at the individual, household, community and national level.

Research Project Name	RACSMEI
Funding	Wellcome Trust
Project duration	2025 - 2030
Collaboration	IPC's Epidemiology Unit (Dr. Claude FLAMAND, Dr. Tephanie SIENG) Malaria Consortium (Ms Mousumi RAHMAN) Medical Biology IPC Laboratory (Dr. Sokhleap CHENG) IPC Virology Unit (Dr. Erik KARLSSON, serological testing, pathogen detection and metagenomic sequencing.) IPC Medical and Veterinary Entomology (Dr. Sebastien BOYER) Institute Pasteur France (Prof Simon CAUCHEMEZ, Dr. Michael WHITE)

WaSPP: Detection of pathogens with pandemic potential in urban wastewater as an early warning of human adaptation and transmission

This project aims to develop and implement a robust wastewater surveillance system to monitor viral families with pandemic potential in Southeast Asia. Focusing on priority pathogen panels, the initiative will optimize protocols for sample concentration and next-generation sequencing, and validate these methods in local laboratories across the region. Pilot surveillance activities will be carried out in Malaysia, Indonesia, and Cambodia. The broader objective is to establish a model for wastewater-based surveillance tailored to pandemic-prone viruses, define a target product profile, and foster a regional network to support knowledge exchange and professional development in pathogen monitoring.

Research Project Name	WaSPP
Funding	Imperial College, HKJC Charities Trust (Institute of Philosophy)
Project duration	2025 - 2027
Collaboration	Institut Pasteur du Cambodge: Virology Unit (EA Karlsson)

Molecular Characterization Hepatis C Virus (HCV) circulating in Cameroun

An understanding of HCV molecular epidemiology is important for surveillance of transmission dynamics and leads to an appropriate public health response. In the era of direct-acting antiviral (DAA) therapy, knowledge of HCV genetic diversity may also be useful for the treatment and management of HCV-infected patients, particularly in case of virological failure. The majority of described HCV infection and treatment outcome is associated with HCV genotype 1, which is globally distributed and well conserved. In contrast, high diversity HCV lineages are observed in high endemic areas such as Africa. The aim of the study is to describe HCV genotypes, subtypes, and potential recombinants circulating in Cameroon between 2013 and 2023, based on HCV capsid and NS5B genes. Additionally, we aim to characterize the complete genomes of novel HCV subtypes and recombinants using ONT sequencing. The study will be conducted in collaboration with Centre Pasteur du Cameroun (CPC). A total of 100 HCV positive samples will be sent from CPC to IPC. A PhD student from CPC will receive a 2-month internship on ONT next generation sequencing in the Virology Unit.

Research Project Name	Molecular Characterization Hepatis C Virus (HCV) circulating in Cameroun
Funding	IPC Internal Funding and Calmette & Yersin Internship Grant
Project duration	2025
Collaboration	Institut Pasteur du Cambodge: Virology Unit (L Pum, EA Karlsson, and J Nouhin); Sequencing Platform (V Heang and J Nouhin) Centre Pasteur du Cameroun (A Mounchili and R Njouom)

ECOMORE 3

As part of efforts to strengthen health security in the Indo-Pacific region, the ECOMORE 3 project will focus on studying the circulation of priority zoonotic and vector-borne diseases in Southeast Asia. Using innovative laboratory diagnostic techniques, the project will integrate data on environmental and climatic factors, while also characterizing vectors and animal reservoirs. The ECOMORE consortium, coordinated by the Institut Pasteur, brings together five key partners across four countries: the Institut Pasteur du Cambodge (IPC) in Phnom Penh, the Institut Pasteur du Laos (IPL) in Vientiane, the National Institute of Hygiene and Epidemiology (NIHE) in Hanoi, and the Research Institute for Tropical Medicine (RITM) in Manila. These national institutes, affiliated with their respective Ministries of Health, play vital roles in their countries' public health systems. The French National Research Institute for Sustainable Development (IRD) will contribute expertise on the climate component of the program.

In Cambodia, a multidisciplinary team—comprising virologists, entomologists, epidemiologists, and climatologists—will adopt a One Health approach to conduct a nationally representative longitudinal survey on zoonotic and endemic diseases. The study will also explore how human activity, land use, and climate change influence the distribution of major vectors in the country. In parallel, novel diagnostic tools will be developed to support research activities and build sustainable capacity.

The project aims to:

(1) strengthen integrated human, animal, and environmental health approaches, and

(2) establish a collaborative framework for epidemic preparedness and response (PPR).

These goals will be implemented across four key areas: Laboratory Capacity, Climate and Health, Surveillance, and Response.

Research Project Name	ECOMORE 3
Funding	AFD
Project duration	2024-2027
Collaboration	Institut Pasteur du Cambodge: Virology Unit (E Karlsson) Institut Pasteur du Cambodge: Epidemiology and Public Health Unit (Claude Flamand and Sowath Ly) Institut Pasteur du Cambodge : Entomology Unit (Sébastien Boyer) Institut Pasteur du Laos, Lao RDP National Institute of Hygiene and Epidemiology (NIHE), Vietnam Research Institute of Tropical Medicine (RITM), the Philippines

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

The primary objectives of this study are to: (1) identify the causative agents of community-acquired pneumonia (CAP), and (2) detect novel viruses or pathogens responsible for CAP. The study will be conducted at Calmette Hospital for adult patients and Kuntha Bopha Hospital for pediatric patients. Respiratory samples will undergo multiplex RT-PCR testing to screen for both viral and bacterial pathogens. A subset of RT-PCR negative samples will be further analyzed using metagenomic sequencing to identify potential novel pathogens. Additionally, serum samples will be tested for IgG antibodies against emerging pathogens using a multiplex serological assay developed by the Singaporean team at the National Centre for Infectious Diseases. The study protocol was submitted to the National Ethics Committee for Health Research (NECHR) in Cambodia for approval in December 2024 and will kick off in 2025.

Research Project Name	CAP-Study
Funding	PREPARE Programme, National Centre for Infectious Diseases, Singapore
Project duration	2024-2026
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Veasna Duong, Erik Karlsson, and Kang Sunheng) Calmette Hospital (Dr. Sotharith Bory) Kuntha Bopha Hospital (Dr. Nguon Yaneth), Cambodoa National Centre for Infectious Diseases, Singapore (Dr. Yeo Tsin Wen)

Preventing Avian Influenza in the Pacific

The PACAI project, funded by the PREZODE initiative and led by the Institut Pasteur de Nouvelle-Calédonie, aims to prevent the emergence and spread of Highly Pathogenic Avian Influenza (HPAI) within Pacific Island Countries and Territories (PICTs). The project will implement advanced surveillance techniques in domestic and wild bird populations and utilize innovative diagnostic tools to enhance detection capabilities. PACAI will also assess the economic and societal impacts of avian influenza, strengthening local preparedness and response strategies. Activities are conducted in close collaboration with regional and international partners, adopting a multidisciplinary One Health approach to safeguard both human and animal health.

The planning phase is underway, with initial fieldwork pending regulatory approvals from local authorities.

Research Project Name	PACAI (Preventing Avian Influenza in the Pacific)
Funding	PREZODE Initiative (France 2030)
Project duration	2025-2027
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson). Institut Pasteur de Nouvelle-Calédonie (M. Dupont-Rouzeyrol), Institut Pasteur Paris (J. Vanhomwegen), Institut de Recherche pour le Développement (IRD) (A. Ponchon, E. Vidal), Institut Agronomique Néo-Calédonien (IAC), Institut Pasteur du Cambodge, Université de la Nouvelle-Calédonie, Communauté du Pacifique, Direction des Affaires Vétérinaires Alimentaires et Rurales, Bird Conservation New Caledonia, Direction des Affaires Sanitaires et Sociales, Centre Hospitalier Territorial.

Development and Assessment of Novel, High-Throughput Immunological Assays for Viral Spillover Surveillance

This project, funded by the UKRI Funding Service and led by the University of Oxford, aims to develop and apply innovative, high-throughput immunological assays to enhance surveillance and early detection of zoonotic virus spillover events in Southeast Asia, a region of significant vulnerability for emerging infections. Immunological tools focusing on antibody and T-cell responses will be established to comprehensively map the immune landscape against critical viral families, including Coronaviridae, Orthomyxoviridae, and Paramyxoviridae, in high-risk populations in Cambodia and Vietnam. The project will assess how immunological surveillance strategies can detect spillover events earlier than traditional methods, thereby strengthening pandemic preparedness and response. Additionally, potent broadly neutralizing antibodies against zoonotic viruses will be identified from previously infected individuals. The project fosters a strong collaborative network involving regional researchers and international experts, enhancing local research capacity and enabling regionally-led responses to future health crises. The planning phase is progressing, with fieldwork scheduled to commence in April 2025, following ethical and regulatory approvals.

Research Project Name	ImmunoSurveil-SEA (Development and Assessment of Novel, High-Throughput Immunological Assays for Viral Spillover Surveillance)
Funding	UK Research and Innovation (UKRI) Funding Service
Project duration	2025-2028
Collaboration	Institut Pasteur du Cambodge (Claude Flamand, Erik Karlsson, Sowath Ly, Tineke Cantaert, Veasna Duong), University of Oxford (Le Van Tan, Gavin Screaton, Nguyen To Anh, Peter Horby), Bioinformatics Institute Singapore (Sebastian Maurer-Stroh), Duke-NUS Medical School (Anthony Tan, Nina Le Bert), Imperial College London (Azra Ghani, Ruth McCabe), National Institute of Hygiene and Epidemiology Vietnam (Pham Quang Thai, Khang Pham Van, Le Hai Dang, Lê Thị Thanh, Nguyễn Phương Anh, Nguyễn Vũ Sơn), National University of Singapore (Chee Wah Tan, Hannah Clapham).

H5N1 Cross-Platform Readiness

This project, funded by Wellcome and led by researchers across Africa and Southeast Asia (SEA), aims to establish a multidisciplinary research platform for the rapid detection and characterization of H5N1 avian influenza infections. By leveraging molecular and immunological tools, the project will enhance

local surveillance capacity, identify human H5N1 infections, and assess population immunity through antibody and T-cell assays. The research will be conducted in collaboration with leading institutions in Africa, SEA, and international partners, ensuring coordinated responses to potential pandemic threats. The project builds upon existing genomic surveillance and immunology research networks, integrating expertise in viral detection, immunological analysis, and public health strategy. Initial field activities are set to begin following the implementation of laboratory capacity-building efforts and regulatory approvals.

Research Project Name	H5N1 Cross-Platform Readiness
Funding	Wellcome
Project duration	2025–2027
Collaboration	Institut Pasteur du Cambodge (Erik Karlsson), Wellcome-funded MIPs and CIDRI-A, University of Oxford (Dong, Dunachie, Screaton), Mahidol University (Dejnirattisai, Chantima), National University of Singapore (Tan), La Jolla Institute for Immunology (Grifoni, Sette), Africa Health Research Institute (AHRI), Kenya Medical Research Institute-Wellcome Trust Research Programme (KWTRP), Mahidol Oxford Tropical Medicine Research Unit (MORU), Oxford University Clinical Research Unit (OUCRU), Malawi-Liverpool-Wellcome Trust Clinical Research Programme (MLW).

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

The objective of the project is to build capacity for use of the new cell culture facility to utilize human and novel chicken cell lines for future risk assessment, diagnostics development, and pandemic preparedness.

Research Project Name	CEIRR Pilot Project: Capacity building for in vitro influenza diagnostics.
Funding	CEIRR/NIAID SUBMITTED IN 2025
Project duration	2025–2026
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson), St. Jude Children's Research Hospital (Stacey Schultz)

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

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

Research Project Name	Assessing the transmission, carrying capacity, and pathogenesis of the avian influenza virus associated with native chicken and duck breeds in the Greater Mekong Subregion
Funding	Wellcome Trust; PENDING – To be submitted July 2025 Food and Agriculture Organization of the United Nations/USAID – Pilot Funded 2024/2025
Project duration	2024–2027
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson) Cambridge (Jim Kaufman) Ben-Gurion University of the Negev (Tomer Hertz)

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

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

Research Project Name	Pilot implementation of the SILAB LIMS system in Cambodia for use in AIV surveillance.
Funding	FAO
Project duration	Started March 2022 through 2024, and to be continued in 2025
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson, Heidi Auerswald) Theramo

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

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

Research Project Name	Scaling up early warning surveillance using novel collection and sequencing techniques.
Funding	Hong Kong Jockey Club, In Discussion 2025
Project duration	2025-2027
Collaboration	Institut Pasteur du Cambodge: Virology Unit (Erik Karlsson) Food and Agriculture Organization of the United Nations (FAO) Hong Kong University Other ministries, stakeholders in partner countries

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

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

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

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.

The study protocol of seroprevalence survey has been submitted to NECHR and is expected to be reviewed on Feb. 26, 2025.

The coordinating team is currently developing the study protocol for the animal component, which will be submitted to the Cambodian Ministry of Agriculture Forestry and Fisheries (MAFF). Field activities will start as soon as approval is obtained from MAFF or Inter Ministerial Coordination Committee for One Health (IMCC-OH).

Research Project Name	PREZODE-AFRICAM
Funding	French Development Agency (AFD)
Project duration	2024-2026
Collaboration	Institut Pasteur du Cambodge: Virology Unit (J Guillebaud, L Khun, T Hoem, S Nuon, V Hul, and J Nouhin), Medical and Veterinary Entomology Unit (S Boyer); Epidemiology and Public Health Unit (S Sorn, C Flammand, and S Ly) IRD (V Herbreteau and A-L Banuls) CIRAD (H Guis)

Pathogen discovey in rodents

Southeast Asia, a global hotspot for emerging infectious diseases, has a tropical climate supporting year-round ectoparasite activity. Economic growth and demand for meat products spur increases in agriculture and livestock. Deforestation brings wildlife populations into greater contact with livestock and people. Cambodia already has an enormously high burden of infectious diseases; however, little is known about hotspots of undetected rodent-borne pathogens. We aim to conduct an explorative study to detect the presence of emerging viruses including SFTSV, henipaviruses, and orthomyxoviruses in rodents collected from various settings of Cambodia including urban, semi-urban, and rural area. This is a retrospective study utilizing samples available in the Virology biobank. Rodent samples will be tested for pathogen using molecular assays including PCR and next generation sequencing.

Research Project Name	Pathogen discovery in rodents
Funding	IPC Internal Funding
Project duration	2025 - 2026
Collaboration	Institut Pasteur du Cambodge: Virology Unit (L Khun, T Hoem, L Pum, S Nuon, EA Karlsson, and J Nouhin)

Workshops

In 2025, the Virology Unit will continue to expand its capacity-building initiatives through a series of specialized workshops focused on pathogen surveillance, sequencing technologies, and environmental monitoring. These training sessions will enhance technical expertise, facilitate technology transfer, and strengthen collaborations with national and international partners.

Planned Workshops and Training Sessions for 2025:

1. Vietnam, March, 2025 – Avian Influenza Virus and African Swine Fever Sequencing Training in collaboration with the National Center for Veterinary Diagnostics
2. Nepal, April, 2025 – Avian Influenza Sequencing Training with the Nepal National Influenza Center
3. Cambodia, May, 2025 – Environmental Surveillance Sampling Training in collaboration with IAEA/FAO
4. Singapore, May, 2025 – Metagenomics Workshop for RESPIRO in collaboration with the Asia Pacific Genomics Institute
5. Durban, South Africa, September, 2025 – Utilizing Metagenomic Data from Wastewater for Public Health Action

These workshops will play a crucial role in strengthening the unit's research capabilities, reinforcing its role in global surveillance efforts, and fostering knowledge-sharing among key stakeholders.

Other Points

The Virology Unit is entering a pivotal phase in its development, requiring a renewed focus on restructuring, expanding research capacity, and securing its long-term position as a leader in infectious disease surveillance and virology research. Several key priorities will guide the unit's growth in 2025, ensuring it remains at the forefront of pathogen monitoring, genomic surveillance, and public health preparedness.

A major initiative for the coming year will be the reorganization of the unit, building on past successes while adapting to new challenges. This will involve refining the unit's vision, communication strategy, and overall branding, ensuring that its mission and impact are effectively conveyed to both scientific and public health audiences. Clearer communication, stronger institutional identity, and strategic outreach will enhance collaboration opportunities, attract funding, and reinforce the unit's role as a key player in global virology research.

To meet increasing demands, the unit must also expand its team, addressing the significant loss of senior scientists in the past year. The departure of experienced researchers has created gaps in leadership, expertise, and project continuity, making it essential to hire additional scientists, postdoctoral researchers, and technical staff. With the rising complexity of research programs and the growing workload, investment in new talent will ensure continued innovation and scientific excellence. Expanding the team will also support the development of new methodologies, maintain high research output, and enhance collaboration with national and international partners.

Another critical objective for 2025 is to increase scientific publications, ensuring that the unit's research contributes to the global virology and infectious disease community. Recent advances in avian influenza surveillance, environmental sampling, viral genomics, and zoonotic disease research have generated a wealth of data that must be disseminated. Strengthening publication efforts will not only improve visibility but also demonstrate leadership in key areas of virology research, attracting funding and fostering stronger collaborations.

Despite ongoing challenges, particularly funding constraints, the unit remains committed to continuing essential projects such as live bird market surveillance. This program is fundamental to understanding avian influenza virus evolution, reassortment, and potential spillover risks, and its continuity is a priority. Similarly, ongoing One Health initiatives focused on rodent-borne and bat-associated viruses will persist, as they are critical for assessing zoonotic threats. Maintaining these

projects, even in the face of financial uncertainty, will require stronger advocacy for funding, collaboration with governmental and non-governmental partners, and efficient resource allocation.

In addition to expanding existing research, the unit will continue to develop and integrate new projects, staying ahead of emerging infectious disease challenges. The integration of advanced sequencing technologies, real-time pathogen detection tools, and environmental surveillance methodologies will be key to improving detection capabilities. The unit will also explore new avenues in pandemic preparedness, viral evolution studies, and AI-driven epidemiological modeling, further strengthening its impact on public health.

Two major unit goals for 2025 will be achieving FAO Reference Laboratory status and obtaining ISO accreditation. These objectives were outlined in last year's strategic outlook and remain central to the unit's long-term vision. Becoming an FAO Reference Laboratory will solidify the unit's role as a regional hub for virological expertise, enhancing its ability to support global surveillance networks and provide critical diagnostic and research support. Likewise, ISO accreditation will improve laboratory standards, ensure compliance with international quality assurance protocols, and enhance credibility in diagnostic and research activities. These milestones will require significant internal restructuring, documentation efforts, and adherence to rigorous quality management standards, but they are necessary to elevate the unit's global standing.

Looking ahead, the Virology Unit is poised to enter a new phase of growth, innovation, and international recognition. Through strategic reorganization, expansion of expertise, increased scientific output, and stronger institutional frameworks, the unit will continue to lead in virology research while reinforcing Cambodia's role in global disease surveillance and preparedness.

The past year has been one of transformation for the Virology Unit. The restructuring process, combined with shifting research priorities and the departure of several senior scientists, has presented both challenges and opportunities. Despite these changes, the unit remains committed to advancing its scientific mission, adapting to evolving public health needs, and reinforcing its role as a regional and global leader in virology research.

To ensure sustainability and further growth, a series of strategic short- and medium-term initiatives will be pursued. Strengthening technical expertise through specialized training and technology transfer, attracting senior researchers and postdoctoral fellows, and fostering interdisciplinary collaborations will be essential to maintaining and expanding the unit's capabilities. Equally critical is the continued publication of the vast amount of data generated, ensuring that findings contribute to global scientific discourse and inform public health interventions.

In the coming years, the unit will build upon its core strengths while strategically expanding into new areas of research and diagnostics. Several key objectives will define this trajectory. The capacity for in-vitro research will be significantly enhanced, including the expansion of a comprehensive biorepository of immortalized and primary cell lines. The implementation of a reverse genetics system for host-pathogen studies, including virus-like particles and pseudoviruses, will allow for deeper mechanistic investigations into viral pathogenesis and immune responses.

The integration of next-generation sequencing and metagenomic sequencing will continue to evolve, with the sequencing mini-platform further streamlined for real-time pathogen detection and evolutionary analysis. These advancements will be accompanied by the strengthening of bioinformatics capabilities, with the recruitment of a dedicated junior bioinformatician to support data analysis, modeling, and visualization.

The unit's serological and molecular diagnostic capacity will expand with the continued development of Luminex assays, which have already been implemented for coronaviruses and arboviruses. These

multiplexed immunoassays will be extended to additional pathogens, bolstering surveillance efforts and supporting research into emerging viral threats.

International collaboration remains a cornerstone of the unit's long-term strategy. Strengthening partnerships within Institut Pasteur Paris, the Pasteur International Network, and global research institutions will facilitate the integration of fundamental research into applied virology, enhancing the unit's ability to translate basic scientific discoveries into public health solutions. Further fostering regional collaboration in emerging infectious diseases will not only enhance visibility but also create new opportunities for funding, joint research projects, and capacity-building efforts across Southeast Asia.

The unit will continue to develop research programs that bridge fundamental and applied science, particularly in mechanistic studies of viral evolution, host interactions, and immune responses. Through these programs, additional postdoctoral fellows and visiting scientists will be brought in to complement ongoing projects, providing fresh perspectives and expertise. The enrollment of students in master's and PhD programs will also be prioritized, both through Cambodian students attending foreign institutions and international students conducting research at the Virology Unit in collaboration with partner universities.

To support these expanding activities, the unit will continue to implement an improved, standardized laboratory information management system, optimizing data management, sample tracking, and diagnostic reporting.

Several major institutional milestones are also on the horizon. Preparations for ISO 17025 accreditation will continue, ensuring that the unit's reference laboratory activities meet international standards. The designation as a FAO Reference Center and a WHO Collaborating Center for Innovation in Emerging and Endemic Virus Surveillance and Response will be finalized, further cementing the unit's role as a hub for virological expertise.

Additionally, the unit seeks to expand its reference laboratory activities, including pursuing WOAH rabies reference laboratory status and broadening rabies serology services to include non-domestic sample testing. Diagnostic capacity will be strengthened with the development and refinement of new serological and molecular assays, along with sustained participation in international proficiency testing programs. These initiatives will position the Virology Unit as a premier reference laboratory for zoonotic research and emerging infectious disease surveillance.

The next three to five years will be transformative, marked by scientific expansion, technological advancements, and deeper engagement with the global virology community. By reinforcing its core research pillars, embracing innovation, and strengthening collaborations, the unit will continue to lead efforts in pathogen surveillance, outbreak response, and virological research, ensuring that it remains at the forefront of infectious disease prevention and control.

3.4.7 Scientific Publications 2024

Note: *The name of authors from the Institut Pasteur du Cambodge are underlined*

1. **A call to innovate Antarctic avian influenza surveillance.**
   Wille M, Dewar ML, Claes F, Thielen P, Karlsson EA.
   Trends Ecol Evol. 2024 Nov 29:S0169-5347(24)00278-7. doi: 10.1016/j.tree.2024.11.005

2. **Bayesian modeling of post-vaccination serological data suggests that yearly vaccination of dog aged <2 years old is efficient to stop rabies circulation in Cambodia.**

Auerswald H, Guillebaud J, Durand B, Le Vu M, Sorn S, In S, Pov V, Davun H, Duong V, Ly S, Dussart P, Chevalier V.
PLoS Negl Trop Dis. 2024 Apr 18;18(4):e0012089. doi: 10.1371/journal.pntd.0012089. eCollection 2024 Apr. PMID: 38635851

3. **Development and field evaluation in African and Asian countries of an hepatitis B virus PCR on open polyvalent platforms to determine treatment eligibility: results from the "Agence Nationale de Recherche sur le Sida et les hépatites" 12327 study.**
D.Kania, J. Nouhin, K. Bolloré, R. Njouom, T. d'A. Toni, A. Issiaka Maiga, C. Toure-Kane, N. Ngo-Giang-Huong, A. Dagnra, D. Hoang Chuong, F. Lunel-Fabiani, J. Castera-Guy, P.
A. Rubbo, A. Pisoni, J.C Plantier, E. Tuaillon
Clinical Microbiology and Infection - Available online 11 May 2024. https://doi.org/10.1016/j.cmi.2024.05.002.

4. **Ebola-specific therapeutic antibodies from lab to clinic: The example of ZMapp.**
Wong G, Bienes KM, Xiii A, Fausther-Bovendo H, Kobinger GP.
Antiviral Res. 2024 Apr 3:105873. doi: 10.1016/j.antiviral.2024.105873. Online ahead of print.

5. **Evaluation of one-year immunity following rabies post-exposure prophylaxis in dog bite cases**
Ya N, Auerswald H, Touch S, In S, Yun C, Thai P, Sann S, Heng B, Leng C, Duong V, Peng YS, Ly S, Cantaert T.
NPJ Vaccines. 2024 Nov 27;9(1):237. doi: 10.1038/s41541-024-01030-8.

6. **Ingrained: Rice farming and the risk of zoonotic spillover, examples from Cambodia.**
Sievers BL, Hyder S, Claes F, Karlsson EA.
One Health. 2024 Feb 29; eCollection 2024 Jun. PMID: 39010950
10.1016/j.onehlt.2024.100696

7. **Pathogen genomic surveillance status among lower resource settings in Asia.**
Getchell M, Wulandari S, de Alwis R, Agoramurthy S, Khoo YK, Mak TM, Moe L, Stona AC, Pang J, Momin MHFHA, Amir A, Andalucia LR, Azzam G, Chin S, Chookajorn T, Arunkumar G, Hung DT, Ikram A, Jha R, Karlsson EA, Le Thi MQ, Mahasirimongkol S, Malavige GN, Manning JE, Munira SL, Trung NV, Nisar I, Qadri F, Qamar FN, Robinson MT, Saloma CP, Setk S, Shirin T, Tan LV, Dizon TJR, Thayan R, Thu HM, Tissera H, Xangsayarath P, Zaini Z, Lim JCW, Maurer-Stroh S, Smith GJD, Wang LF, Pronyk P; Asia Pathogen Genomics Initiative (Asia PGI) consortium.
Nat Microbiol. 2024 Sep 24. doi: 10.1038/s41564-024-01809-4.

8. **Simplified Criteria to Assess Long-Term Antiviral Treatment Indication in Chronic HBV-Infected Pregnant Women in Cambodia**
J.S. Yang, S. Sovann, Y. Shimakawa, S. Nhoueng, B. Dim, C.Vong, C. Sann, J. Guillebaud, D. Vann, B. Touch, H. Chea, W. Pisey Choupoan Phirum, E. Rosenthal , C. Paul, L. Khun, C. Yay, D. Laurent, S. Chhun, L. Borand and O. Segeral**
Viruses 2024, *16*(2), 194; https://www.mdpi.com/1999-4915/16/2/194

9. **"Smart markets": harnessing the potential of new technologies for endemic and emerging infectious disease surveillance in traditional food markets**
   Benjamin L. Sievers, Jurre Y. Siegers, Jimmy M. Cadènes, Sudipta Hyder, Frida E. Sparaciari, Filip Claes, Cadhla Firth, Paul F. Horwood, Erik A. Karlsson
   Journal of Virology 16 January 2024 DOI: https://journals.asm.org/doi/10.1128/jvi.01683-23

10. **Detection and phylogenetic analysis of contemporary H14N2 Avian influenza A virus in domestic ducks in Southeast Asia (Cambodia)**
    Siegers JY, Wille M, Yann S, et al.
    Emerging Microbes & Infections. 2024; 13(1):2297552. doi: 10.1080/22221751.2023.2297552.

11. **Diet-induced obesity affects influenza disease severity and transmission dynamics in ferrets**
    Meliopoulos V, Honce R, Livingston B, et al.
    Science Advances. 2024; 10(19):eadk9137. doi: 10.1126/sciadv.adk9137.

12. **Spatiotemporal evolution and transmission dynamics of Alpha and Delta SARS-CoV-2 variants contributing to sequential outbreaks in Cambodia during 2021**
    Su YCF, Zeller MA, Ou TP, et al.
    Communications Medicine. 2024; 4(1):252. doi: 10.1038/s43856-024-00685-7.

13. **African swine fever virus genotype II Eurasian spatiotemporal expansion and its origin and spread in Cambodia**
    Thézé J, Hidano A, Tum S, et al.
    Virus Genomics, Evolution and Bioinformatics. 2024.

14. **Emergence of a novel reassortant clade 2.3.2.1c avian influenza A/H5N1 virus associated with human cases in Cambodia**
    Siegers JY, Xie R, Byrne AMP, et al.
    medRxiv. 2024 Nov 04. doi: 10.1101/2024.11.04.24313747.

15. **The genetic diversity of Nipah virus across spatial scales**
    Cortes-Azuero O, Lefrancq N, Nikolay B, et al.
    Journal of Infectious Diseases. 2024 Apr 29. doi: 10.1093/infdis/jiae221.

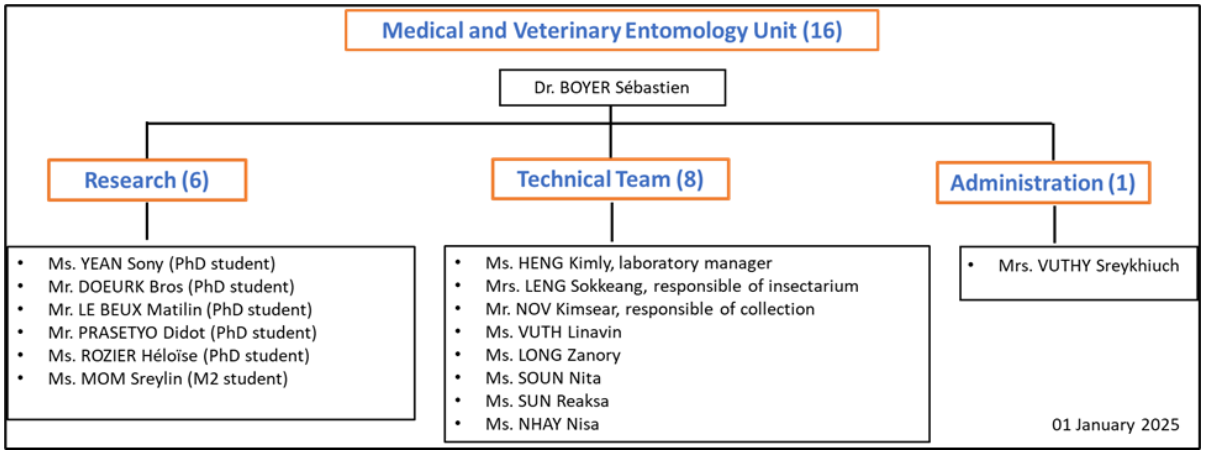
16. **Viromes of arthropod parasites and their hosts: The case of bats and bat ectoparasites**
    Tendu A, Li R, Kane Y, et al.
    Acta Tropica. 2024 Sep 1;259:107375. doi: 10.1016/j.actatropica.2024.107375.

17. **We need a global framework for promoting safe handling of high-consequence pathogens**
    Karlsson EA, Blacksell SD, Carroll D, et al.
    BMJ. 2024 Aug 23;386:q1855. doi: 10.1136/bmj.q1855.

3.5 Medical & Veterinary Entomology Unit

3.5.1 Functional Structure

The Medical and Veterinary Entomology Unit was officially established on October 1, 2018 (reference: N°413/IPC/DIR/2018), coinciding with the recruitment of Dr. Sébastien BOYER by *Institut Pasteur* in Paris for a permanent position. The unit's staff comprises 16 members.



3.5.2 Research Program - Major Achievements in 2024

3.5.3.1. Overview

Human Resources Movement

Dr. RAKOTONIRINA Antsa, a post-doctoral researcher, completed her 3-year contract in April 2024 and joined the Institut Pasteur de Madagascar for a PSRL position. In addition, four experienced and skilled technicians left at the beginning of 2024: Mr. SUOR Kimhuor, responsible for entomological collections, and Ms. KRIB Davy were both successful in the recruitment exam for the Ministry of Environment. Ms. SEN Saoya passed the recruitment exam for the ministry of Education, Youth and Sport. Finally, Ms. CHEAR Rina joined an American NGO working on malaria mosquitoes.

In 2024, we recruited four new technicians (Ms. LONG Zanory, Ms. SOUN Nita, Ms. SUN Reaksa and Ms. NHAY Nisa) to strengthen the team as new projects began. In parallel, as outlined in Section 4.5.1 on the Functional Structure, two PhD students also started their programs in 2024. For 2025, we plan to recruit three post-doctoral researchers and five additional technicians.

Field Missions in 2024

During 2024, the Medical and Veterinary Entomology Unit conducted **25 field missions** in Cambodia (compared to 58 in 2023, 39 in 2022 and 35 in 2021), totaling **367 mission days** in the field (compared to 539 in 2023, 399 in 2022 and 393 in 2021).

6th International Conference on the Tiger Mosquito, *Aedes albopictus*

Our Unit was honored to organize the 6th International Conference on the Tiger Mosquito, Aedes albopictus, on March 28 and 29, 2024 in Phnom Penh. In the absence of a vaccine strategy, the fight against vector species is decisive, requiring a good knowledge of the vector. The conference was therefore an important opportunity to share knowledge about Ae. albopictus. Several researchers gave a variety of presentations, offering diverse perspectives on their work relating to this mosquito species. The first day of the conference featured presentations on the impact of Dengue vector species in Southeast Asia, covering their distribution, biology, ecology, genetics and vector competence. The

second day was dedicated to vector surveillance, insecticide resistance and new vector control strategies.
Internationally recognized scientists from 21 different countries were able to exchange knowledge and, new discoveries, participate in discussions, develop new, innovative and adapted strategies, and strive for quality collaborations. Organized every two years on an international scale, this conference has already been held in China (Guangzhou, twice), Italy (Pavia, twice) and France. This is the first time the event has been held in Southeast Asia, in Cambodia to be precise.

Pasteur Network entomological scientists

Our Unit organized a working group of entomologist scientists from the Pasteur Network at the IPC. This was an opportunity for researchers to present their current activities, possible future projects and explore possibilities for collaboration. Entomologists present were from Instituts Pasteur Paris, Italy Fondation Cenci-Bolognetti, Bangui, Tunis, Ho Chi Minh City, Lao and of course Cambodia.

Scientific projects

In 2024, NIH-PICREID and ANR-BCOMING projects continued, while the FSPI-VECAM project concluded. Two projects, FEF-R CISED and FEF-R Photo, began in August, and the ANRS-MIE SEAROADS project started in October. Looking ahead to 2025, the projects ICEMR Malaria, AFD-AFRICAM and WT-RACSMEI are scheduled to begin. Additionally, the ECOMORE 3 project is planned to start at the end of 2025.

3.5.3.2. List of projects

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

The PICREID project aims to establish a "One Health" approach to enhance the capacity for rapid and effective responses to emerging infectious diseases outbreaks in Southeast Asia. The surveillance enhancement component of the PICREID project is based on RNA virus detection, understanding the transmission of endemic RNA viruses, identifying factors that influence RNA adaptations to new hosts, and studying the adaptive responses of emerging infectious diseases.
The mosquito component seeks to study the dynamics of the main dengue virus vector species in Kampong Thom Province (*Aedes aegypti* and *Aedes albopictus*), describe mosquito behavior, characterize their ecology, and further analyze and model their spatial distribution and the effects of land-use on their dynamics. Another objective is to model dengue risk by correlating the number of dengue vectors with the number of dengue cases in humans. Additionally, the project integrates pathogen discovery with entomological genomic surveillance in collaboration with the *Institut Pasteur* in Paris.
In 2024, eight field missions were conducted. Six of these missions focused on analyzing the dynamics of relative mosquito densities in Kampong Thom Province in relation to different types of land use. Two missions were dedicated to larval collection for identifying breeding sites.

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

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

The project will analyze how biodiversity impacts the risk of infectious disease emergence and aims to develop tools for biodiversity conservation and restoration strategies to reduce zoonotic risk. If feasible, surveillance and pathogen detection strategies will be implemented.

Biodiversity loss in hotspots is critical to understanding and preventing future pandemics. The COVID-19 crisis highlighted limitations in implementing 'One Health' approaches, particularly the lack of context-adapted solutions for stakeholders. To address this, BCOMING will build on past international projects to co-develop innovations with stakeholders in biodiversity hotspots, reducing zoonotic risk through conservation and surveillance strategies.

The project's strategies will be implemented in Europe and tropical biodiversity hotspots in Southeast Asia, West Africa, and the Caribbean. BCOMING will enhance understanding of biodiversity's role in disease emergence and develop participatory tools for context-adapted conservation. Its detection and surveillance strategies aim to prevent epidemics from escalating into pandemics. With a strong multi-actor consortium and integration into the PREZODE Initiative, the project will scale up innovations and disseminate socio-economic and environmental strategies globally.

Field missions began in 2023. In 2024, the Unit participated in five missions in Stung Treng Province, in addition to four missions conducted in 2023. During these missions, bats were captured at dusk on three karst mountains—Chhgnauk, Ka Ngoark, and Chab Pleurng—as part of a longitudinal study. Ectoparasites, including Nycteribiidae, Streblidae, Arachnida (mites, ticks), and bat bugs, were collected with forceps directly from the bats' bodies and stored in Eppendorf tubes containing alcohol. To date, over 1,400 ectoparasites have been collected on bats or in the bat caves. In addition to collecting bats' ectoparasites, CDC light traps were used to capture mosquitoes and phlebotomines (sand flies). To date, over 12,800 phlebotomines were collected and belongs to the 2 main families Phlebotomus and Sergentomyia. All specimens obtained during the missions were analyzed in the Unit's laboratory, with most identified to the species level. Our research focuses on the taxonomy of these insects and the host-parasite specialization between bat flies and bats in Cambodia.

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) 2023-2026

Veterinary Entomology in Cambodia (VECAM)

In recent decades, we have witnessed the emergence of several pandemics, the expansion or re-emergence of historical pathogens, and shifts in the ranges of major vectors, raising concerns about the potential emergence of new vectors and epidemics, often at the human-animal interface (i.e., zoonoses). For several years, Cambodia has faced epizootic diseases that have significantly impacted its cattle, sheep, and poultry production sectors. These diseases are primarily transmitted by ticks, fleas, or mosquitoes. However, aside from mosquitoes, the country lacks fundamental information on these vector species, their distribution across the national territory, and effective control methods.

To address this, Cambodia must establish baseline data to monitor and control emerging epidemics. Equally vital is the need to strengthen the capacity of Cambodian technical, academic, and research sectors involved in arthropod vector management to safeguard public and veterinary health.

The overall objective of this project was to develop expertise in veterinary entomology, a field that previously did not exist in Cambodia. The staff who directly benefited from the project were from the four partner organizations: the Institut Pasteur du Cambodge (IPC), the Royal University of Agriculture (RUA), and two institutions under the Ministry of Agriculture, Forestry and Fisheries (GDAHP and NAHPRI).

The project successfully enabled the development of new medium- and long-term surveillance and control methods, as well as diagnostic tools. It also raised awareness among livestock farmers about arthropod vectors in Cambodia.

By forming a unique working group that brought together stakeholders from three different ministries, the FSPI project created a sustainable development think tank to address zoonotic diseases in relation to the environment within socio-ecological systems. In doing so, the project significantly strengthened the One Health approach in Cambodia, fostering reinforced Franco-Cambodian cooperation on these critical health issues.

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

Comprehensive Integrated Surveillance and Early Detection System for Dengue (FEF-R CISED)

In 2023, Cambodia experienced a major dengue outbreak, highlighting the need for improved surveillance and management of vector-borne diseases. Cambodia's environment supports the proliferation of Aedes mosquitoes, the primary dengue vectors. Current surveillance systems, like those from the National Center for Parasitology, Entomology, and Malaria Control (CNM) and the CDC, have limitations, especially in early outbreak detection. The "Comprehensive Integrated Surveillance and Early Detection System for Dengue (CISED-Dengue)" project addresses these gaps by integrating data from multiple sources, including epidemiological, virological, entomological, and environmental information. Collaborating with institutions such as IRD, CDC, MoH, CNM, and IPC, CISED-Dengue enhances real-time understanding of dengue dynamics, vital for effective response to future outbreaks.

The project aligns with the WHO's EWARS framework, using a collaborative approach to share data and foster knowledge exchange. Its interactive dashboard supports real-time analysis and decision-making, helping adapt strategies quickly during outbreaks. CISED-Dengue will develop a digital dashboard with public and professional interfaces, integrating diverse data types for comprehensive monitoring. It will also establish a platform for transparent data sharing between key institutions, including CDC, CNM, IPC, and IRD. Training sessions will be provided to health professionals to enhance their capacity to use data effectively in decision-making.

The project aims to improve the early detection of dengue outbreaks, enabling more informed decisions for public health interventions. It will also raise public awareness, fostering greater engagement in dengue prevention efforts.

Collaborations	*Ambassade de France au Cambodge*, Cambodia Centers for Disease Control and Prevention (CDC), Ministry of Health National Center for Parasitology, Entomology and Malaria Control (CNM), Ministry of Health Institut de Recherche pour le Développement (IRD), GeoHealth
Funding	*FEF-R - Fond Equipe France Rapide*, Paris, France 2024-2025

An Innovative New Tool in Cambodia: Toward a Global Revolution in Imaging for Health Sciences (FEF-R Photo)

The Institute Pasteur in Cambodia (IPC), in collaboration with Cambodia's Ministry of Environment and other government bodies, is developing new tools and expertise to raise awareness about the importance of biodiversity. The Medical and Veterinary Entomology Unit at IPC has made significant contributions, including mosquito species identification, public outreach through local media, and educational materials in multiple languages. A notable achievement is the publication of an insect encyclopedia aimed at children, published in Khmer, French, and English, with a focus on biodiversity education.

The unique photographic technique allows for the detailed depiction of species, unveiling new aspects of biodiversity previously unseen. This innovation led to the development of a world-first functional prototype for insect photography, achieving unprecedented resolution. Initial tests on human liver cells have shown promising results, further validating its potential for scientific and health applications.

The project's development will consist of three main activities: (1) the installation and setup of the equipment at IPC and the training of relevant stakeholders, (2) field missions to capture high-quality photographs of specimens, providing new insights into biodiversity, and (3) testing the method on various species, including animals, plants, and cells. The final phase will focus on communication efforts to spread awareness of the biodiversity crisis and the potential of this new technology in scientific and public health applications.

Collaborations	*Ambassade de France au Cambodge*, Cambodia Institut Français du Cambodge
Funding	*FEF-R - Fond Equipe France Rapide*, Paris, France 2024-2025

One Health Regional Approach for Integrated and Interconnected Urban Dengue Surveillance Southeast Asia (SEAROADS)

Mosquitoes transmit many major infectious diseases, including malaria and arboviral diseases such as dengue, Zika, chikungunya, and yellow fever. Global warming is increasing the burden of vector-borne diseases, enabling their spread from tropical regions to temperate zones. Autochthonous transmission of dengue in southern France exemplifies this threat. Urban adaptation of vectors like *Aedes aegypti* and invasive species such as *Aedes albopictus* heighten public health risks, especially as global temperatures rise. In Southeast Asia, decades of regular dengue epidemics offer an opportunity to understand the dynamics of urban mosquito-borne diseases and develop mitigation strategies applicable to Europe, where arboviruses may become endemic.

This project addresses three key challenges: (i) understanding the intra-urban spatial distribution of mosquito-borne pathogen risk, (ii) improving inadequate entomological surveillance, and (iii)

enhancing surveillance tools at local, national, and regional levels. A multi-scale approach will integrate human mobility and mosquito habitat suitability under climate change scenarios to predict and mitigate dengue risk.

At the intra-urban scale, an agent-based model incorporating mobility and innovative mosquito indices will be developed and calibrated for Bangkok. This model will be extrapolated to major Southeast Asian cities and validated with local data. Scaling up to national and regional levels, coarser human mobility and dengue incidence data will inform surveillance enhancements. A regional platform will be created to connect national systems into an interoperable Early Warning System, integrating satellite-based environmental data.

The project aims to produce a globally implementable prediction and surveillance system to enable rapid public health responses to emerging mosquito-borne diseases. It aligns with EMERGENCE PRFI 2023 objectives by advancing knowledge on emerging diseases, fostering cross-disciplinary collaboration, and standardizing surveillance tools to model and predict epidemic dynamics.

Collaborations	*Ambassade de France au Cambodge*, Cambodia Centers for Disease Control and Prevention (CDC), Ministry of Health IRD (GeohEalth), Cambodia KHEOBS - ITC, Cambodia NCLE, MoH, Lao PDR VCC-SEA, Lao PDR IRASEC, Thailand Mahidol University, Thailand IP HCMC, Vietnam NIMPE, Vietnam Institut Pasteur, France IDEES (UMR CNRS IDEES), Université de Rouen, France
Funding	*ANRS-MIE*, Paris, France 2024-2027

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 2024.

Several different taxonomy projects were initiated and continued in 2024:

- Creation of a MALDI-TOF MS database for mosquito species

Since 2020, we have made efforts to develop a method using MALDI-TOF MS. The current methods for mosquito identification include both morphological and molecular methods. Identification by morphology is skill-dependent and is time-consuming while the identification by PCR is expensive. The MALDI-TOF MS technology, now routinely used for bacterial identification, has recently emerged in the field of entomology. At the end of 2022, 211 individual mosquitoes and 3,424 spectra were implemented.

- Barcoding of mosquito species of Cambodia

Barcoding of COI genes is in progress. Up until now, 111 mosquitoes belonging to 24 species have been barcoded, or sequenced. We have sequenced the DNA of *Aedes unalom,* a new species of the Scutellaris Group recently recorded in Cambodia. Their DNA sequences were analyzed alongside those of other Scutellaris Group species from Asia and the Pacific islands. The results suggest that the

speciation of *Aedes* species within the Scutellaris Group may be driven by diversity in mammalian hosts, climate and environmental changes, or geological dynamics rather than human migration.

- Identification key for tick species

As previously mentioned in the SEA TICKEY project, the identification key for tick species (around 97 species) in Southeast Asia is in progress. The difficulty in obtaining tick specimens limits the key's accuracy for species identification. Our objective is to send a preliminary identification key to partners in all Southeast Asian countries to test the key and obtain their feedback. A MALDI-TOF MS database for tick species was also launched. It comprises 133 spectra of 16 species. More species will be added in the future. The barcoding using COI is also in progress.

- Identification key for mosquito species

At the same time, we also wanted to develop an identification key for the 290 mosquito species described until now in Cambodia. However, completing this identification key would take a lot of time, especially if done concurrently with the key for tick species. Work on this project began in 2022. We aim to obtain a final key by 2024.

- Streblidae and Nycteribiidae (bat flies)

Since last year, and with the upcoming BCOMING project, we have been collecting bat ectoparasites. Our objective is also to develop a key for these species, and to determine the number of species, the exchanges between these species, and the pathogens they carry. We have also developed COI and MALDI-TOF database for those specimens.

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

3.5.3 Research Programs - Outlook for 2025

AFD-AfriCam Preventing Zoonotic Diseases Emergence (PREZODE)

Through the PREZODE Initiative, Cambodia is the only Asian country involved in the AfriCam project, alongside four African countries: Cameroon, Guinea, Madagascar, and Senegal. This project, running from 2022 to 2024, aimed to study how hydrological dynamics, climate, and environmental factors influence the risk of zoonotic disease emergence across diverse ecosystems. These ecosystems represent key interfaces between humans, animals, and the environment.

Another goal of the project was to implement measures that reduce zoonotic risks. This included strengthening existing surveillance systems in coordination with local and national partners. These efforts are expected to pave the way for an integrated One Health surveillance system in the future.

The AfriCam project was divided into three main components. First, it focused on assessing the risks of zoonotic disease emergence. Second, it investigated the environmental and climatic influences on these risks. Lastly, it established preventive strategies to reduce the likelihood of zoonotic disease emergence. In doing so, the project aimed to enhance surveillance systems and move towards a more integrated One Health approach.

International Center of Excellence in Malaria Research (ICEMR)

In Mondulkiri, two villages located just 5 kilometers apart displayed striking differences in malaria transmission. One village reported active malaria cases, while the other, which was once a hotspot, reported none. Both villages are surrounded by forest and separated by a deforested transitional zone. This contrast provided a unique opportunity to study malaria ecology and control strategies.

The project analyzed vector populations in these two villages, focusing on species diversity, density, and Plasmodium carriage. The proximity of rice fields to one of the villages allowed researchers to explore how forest vectors adapt to agricultural environments, a pattern observed in other regions like Madagascar. These findings were critical in developing malaria elimination strategies, particularly in addressing gaps in vector control and identifying risks for re-emergence in transitional habitats.
The study also examined mosquito biting behaviors to assess human exposure risks and analyzed seasonal patterns of Plasmodium prevalence. Researchers identified the ecological and behavioral roles of Anopheles species in malaria transmission. Additionally, the study evaluated the limitations of current vector control tools and investigated the role of rice fields as breeding grounds for mosquitoes. This provided insights into how vectors adapt to changing environments, contributing to malaria risk.

Welcome Trust- RACSMEI

This project focused on developing a multidisciplinary and cost-effective Precision Public Health framework to address significant knowledge gaps in Cambodia. Cambodia is recognized as a hotspot for zoonotic and vector-borne diseases, including dengue, Japanese encephalitis, avian influenza, and leptospirosis. Adopting a One Health perspective, the study integrated data from humans, animals, vectors, and the environment to assess 11 priority pathogens identified by Cambodian health authorities. A nationwide cross-sectional survey was conducted among 10,000 individuals and their associated ecosystems. Advanced tools such as multiplex serology, metagenomics, and environmental sampling were utilized.
The project aimed to map seroprevalence, understand transmission dynamics, and identify the spatial and behavioral determinants of disease. By linking environmental factors with disease distribution, the project aimed to provide culturally appropriate, targeted intervention strategies. The expected outcomes included generating precise burden estimates for zoonotic and vector-borne diseases, identifying transmission determinants and hotspots, and developing predictive models for pathogen diversity and risk. The project also aimed to propose evidence-based recommendations for intervention strategies.
For vector collection, sentinel BG traps (Biogents AG, Germany) were deployed in selected villages. Traps were placed in ten randomly selected households per village. Mosquitoes collected were identified in the field at the genus level and later confirmed in the laboratory at the species level. This approach generated valuable data on species composition, abundance, distribution, and vector potential, enhancing understanding of vector-borne disease transmission determinants in Cambodia.

AFD-ECOMORE 3

The entomological work package of the Ecomore 3 project in Cambodia aimed to study vector-borne disease transmission in relation to entomological indices and land use. The goal was to support national health authorities in developing effective vector control strategies.
The specific objectives included identifying and characterizing mosquito vector species and analyzing the impact of land use on these species. At the regional level, the project focused on understanding how human activities, land-use changes, and climate change influence the distribution of mosquito vectors across Southeast Asia.
The project aimed to model the transmission dynamics of vector-borne diseases to support the development of a regional health surveillance system. Specific regional objectives included studying the impact of climate and climate change on mosquito species and analyzing how land-use changes

affect vector distribution. The project also sought to standardize mosquito capture methods across the region, improving surveillance and control efforts.

3.5.4 Support to National Authorities

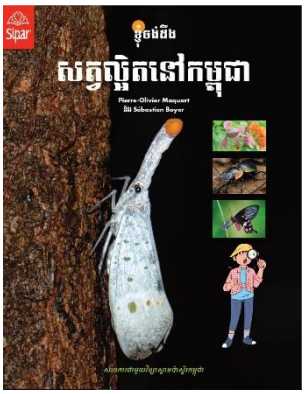
The Medical and Veterinary Entomology Unit is collaborating with five different ministries: the Ministry of Health, the Ministry of Education, Youth and Sport, the Ministry of Agriculture, Forestry and Fisheries, the Ministry of Environment, and the Ministry of Cults and Religion.

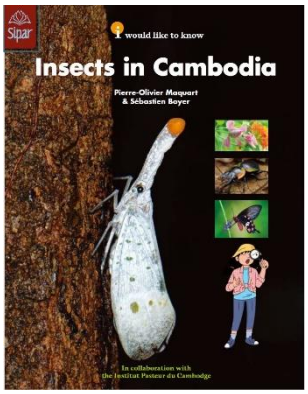
At the request of the Ministry of Agriculture, Forestry and Fisheries, and with the support of the Royal University of Agriculture, two projects were developed and submitted to establish and expand veterinary entomology capabilities in Cambodia. Project VECAM's sole purpose is to support national institutions. 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, which also includes the purchase of equipment for these two institutions. This program is funded by the French Embassy. The second project, mentioned earlier, aims to develop a determination key for tick species. The new project in 2024, FEF-R CISED, is led by the CDC-MoH, and we are supporting their initiative. The SEAROADS project has been developed for the Ministry of Health in Cambodia, as well as for the Ministries of Health in Laos, Vietnam, and Thailand. The project was co-constructed directly with them, and representatives from all involved ministries were present at the KOM to define the main objectives and methodologies.

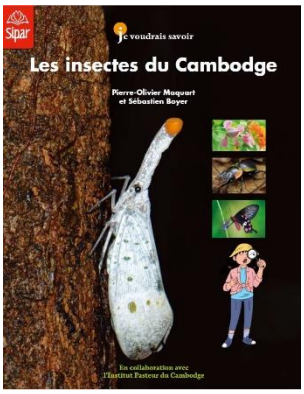
Finally, the upcoming RACSMEI project, led by the Epidemiology Unit, aims to develop risk maps for Cambodia and involves three departments from three ministries as direct co-investigators. These ministries have been involved in the project since its inception.

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

Our Unit published a book to the destination of the children and confirms its commitment to civil society. We realized an educational scientific project, for everyone, especially aimed at young people. As a result, the captivating book entitled "Insects of Cambodia" was published in January 2024. This book, published in three languages (Khmer, French, English), is primarily intended for young minds aged 8 to 12. The lack of scientific books in the Khmer language is often criticized. Our Unit, in collaboration with SIPAR editions, has successfully met this challenge through this publication. We led this project, carefully selecting the most iconic species in Cambodia to be grouped in this comprehensive and didactic work. A cocoon of general information has also been woven to explain the life cycles and peculiarities of these fascinating species.







3.5.5 Teaching and Training

Mentorship

Master's Students (2024)

- **Mr. CABON Flavien** (Master of Biodiversity, Ecology and Evolution, Paul Sabatier University), September 2023 – August 2024
  M2 thesis title: *Bat's ectoparasites of Cambodia* (six months) / *Study of disease vectors along a longitudinal gradient from the large city of Battambang to the floating and flooded cities of the south of the country* (six months).
- **Mr. THORN Vattana** (bachelor of Computer Science in Department of Computer Science, at the Faculty of Digital Engoinneering, CADT, Phnom Penh, Cambodia), January-July 2024
  Thesis title: *TickID*.
- **Mr. ROUXEVILLE Mahé** (Data Science et Modélisation Statistique, Université Bretagne Sud, France), January-August 2024
  M2 thesis title : *Analysis of Dengue and anthropogenic factors at the global scale*.
- **Ms. VUTH Linavin** (Master of Life Sciences and Health, Université Paris-Saclay, Paris, France), May-July 2024)
  M1 thesis title: *Seroprevalence of Tick-Borne diseases on cattle serum*.

PhD Students

- **Ms. YEAN Sony**, Cambodian national, October 2022-September 2025, Doctoral school SDSV of Paris-Saclay (SDSV = Structure et dynamique des systèmes vivants), France
  PhD title: *Tick Species and Tick-Borne Diseases in Cambodia*.
- **Mr. DOEURK Bros**, Cambodian national, October 2023-September 2026, Doctoral school SDSV of Paris-Saclay (SDSV = Structure et dynamique des systèmes vivants), France
  PhD title: *Bionomic of dengue vector mosquitoes in Cambodia*.
- **Ms. ROZIER Héloïse**, French national, October 2023-September 2026, Doctoral school MathSTIC Bretagne Océane, Université de Rennes, France
  PhD title: *Statistical distribution of mosquitoes in Cambodia*.
- **Mr. LE BEUX Matilin**, French national, October 2024 – September 2027, Doctoral school BIOSPC (Bio Sorbonne Pasris Cité), Paris, France
  PhD title: *Machine learning application in Medical Entomology : determining potential biomarkers in mosquito and human for Dengue and Chikungunya viruses*.
- **Mr. PRASETYO Didot**, Indonesian national, October 2024 – September 2027, Doctoral school SFS (Sciences Fondamentales Santé), Université de Reims Champagne-Ardenne (URCA), France.
  PhD title: *Neglected ectoparasites in Cambodia and impact for Public Health.*

Teaching

Since 2020, Sebastien BOYER has been responsible for the "*Vector Borne Diseases and Vector Transmission*" module within the second year of the International Joint Master's in Infectiology: Biology of Infectious Diseases. The module represents 2.5 ECTS credits and consists of 20 hours of instruction. The module was created in 2020, requiring significant time to prepare and complete. It consists of 8 lectures, each lasting 1.5 hours. Our team also conducts the examinations and grading.

Training

In 2024, all newly recruited staff completed the online "Aptitude training course on laboratory security and good laboratory practices".

Additionally, one training session (compared to 9 sessions last year) was provided this year:

11-16 November 2024 **Masterclass on Macro Photography of Insects**
Trainers: Mr. Nathanaël Maury and Ms. Somchit Sudavanh
Trainees: 2 staff members from IPC (FEF-R Photo)

3.5.6 Outlook for upcoming 3-5 years

Within 3 years

- Three long-term projects
- Recruitment of 3 post-doctoral researchers (see priority profiles below)
- Thesis defenses of five PhD students in Medical and Veterinary Entomology
- Development of more mechanistic research (see topics below)
- Mobility of the head of the unit

Within 5 years

- New insectarium and additional space (laboratory and office)
- Future research will depend on the new Head of Unit
- Strengthening the Veterinary and Medical Entomology themes
- Consolidating vector biology research on bat ectoparasites

3.5.6.1. Recruitment

Prioritize filling the identified roles through clear job descriptions and collaborations with universities.

Needed Profiles

- Ecologist
- Vector Competence Specialist
- Taxonomist
- Bioinformatic Specialist
- Molecular Scientist
- Project Manager
- Data Manager
- Statistician

3.5.6.2. Research Focus

Align ongoing and future projects with the thematic listed, ensuring a balance of fieldwork, lab experiments, and computational analysis.

Priority Scientific Thematic

- Pathogen Discovery
- Vector Competence
- eDNA on Mosquitoes
- Insecticide Resistance
- Vector Control Innovations
- Microbiota Research

Develop vector control and insecticide resistance

- **Focus on Vector Control and Prevention**
  Target priority vectors, such as those of dengue and Japanese encephalitis virus (JEV), with research and actionable control measures.
  Investigate and implement alternative control methods (e.g., Wolbachia, microbiota manipulation).
- **Develop a Surveillance System**
  Design a robust, tech-enabled system for real-time vector and disease monitoring.
  Use data from fieldwork and environmental studies to improve prediction and control strategies.
- **Alternative Vector Control Strategies**
  Research innovative methods beyond traditional insecticides, including genetic and

3.5.6.3. Recruitment Capacity Building

Invest in training for existing staff in emerging methods (A.I., MinION) and technologies (bioinformatics).

Methods

- Molecular Techniques (e.g.MinION multiplex sequencing for high-throughput data).
- Cell Culture (P2-Ps3) studies with Virology Unit
- AI and Photo-based Identification
- Bioinformatics
- New data management tools

Human resources

- Organize internal and external training sessions on priority topics (R, GIS, molecular techniques) Increase efforts to attract and retain talent (better salaries, clear career growth opportunities)
- Define clear roles and responsibilities within the unit
- Establish a systematic process for internal knowledge-sharing and meetings.
- Strengthen partnerships with students and public health professionals through workshops, internships, and shared projects.
- Encourage multi-disciplinary skill-building (e.g., combining ecology with molecular biology).

3.5.7 Scientific Publications 2024

Note: *The name of authors from the Institut Pasteur du Cambodge are underlined*

1. **A genotyping array for the globally invasive vector mosquito, Aedes albopictus**
   L. V. Cosme, M. Corley, T. Johnson, D. W. Severson, G. Yan, X. Wang, N. Beebe, A. Maynard, M. Bonizzoni, A. Khorramnejad, A. Jesus Martins, J. B. Pereira Lima, L. E. Munstermann, S. N. Surendran, Chun-Hong Chen, K. Maringer, I. Wahid, S. Mukherjee, J. Xu, Michael C. Fontaine, E. L. Estallo, M. Stein, T. Livdahl, P. Y. Scaraffia, B. H. Carter, M.Mogi, N. Tuno, J. W. Mains, K. A. Medley, D. E. Bowles, Richard J. Gill, R. Eritja, R. González-Obando, H. T. T. Trang, S. Boyer, A. M. Abunyewa, K. Hackett, Tina Wu, J. Nguyễn, J. Shen, H. Zhao, J. E. Crawford, P. A. & Adalgisa Caccone.
   Parasites & Vectors volume 17, Article number: 106 (2024) Published: 04 March 2024 https://doi.org/10.1186/s13071-024-06158-z

2. **Aedes unalom sp. nov. in Cambodia, a new Stegomyia species close to Aedes albopictus (Diptera, Culicidae)**
   M. Hide, P.O Maquart, S. Yean, K. Suor, K. Heng, S. Sen, C. Yim, S. Leng, A. Rakotonirina, D. Fontenille, S. Boyer
   Journal of Asia-Pacific Entomology, Volume 27, Issue 2, June 2024, 102233 https://doi.org/10.1016/j.aspen.2024.102233

3. **Challenges for ticks and tick-borne diseases research in Southeast Asia: Insight from the first international symposium in Cambodia**
   S. Yean, D. B. Prasetyo, S. Marcombe, U. K. Hadi, A.R. Kazim, S. Tiawsirisup, V. Duc Chinh, K. Matsuno, V. Lun Low, S. Bonnet, N. Boulanger, T. Tsan-Yuk Lam, M. Yazid Abdad, V. Herbreteau, J.M. Chavatte, S. Sum,Theary Ren, A. Sakuntabhai, P.O Maquart, A. Rakotonirina,S. Boyer.
   PLoS Negl Trop Dis PMCID: PMC11236135 DOI: 10.1371/journal.pntd.0012269

4. **Metabolic Resistance and Not Voltage-Gated Sodium Channel Gene Mutation Is Associated with Pyrethroid Resistance of *Aedes albopictus* (Skuse, 1894) from Cambodia.**
   Marcombe S, Doeurk B, Thammavong P, Veseli T, Heafield C, Mills MA, Kako S, Prado MF, Thomson S, Millett S, Hill T, Kentsley I, Davies S, Pathiraja G, Daniels B, Browne L, Nyamukanga M, Harvey J, Rubinstein L, Townsend C, Allen Z, Davey-Spence C, Hupi A, Jones AK, Boyer S.
   Insects. 2024 May 15; https://doi.org/10.3390/insects15050358 PMID: 38786914

5. **Mitochondrial and microbial diversity of the invasive mosquito vector species Culex tritaeniorhynchus across its extensive inter-continental geographic range**
   C. L. Jeffries, L. M Tantely, P. Kadriaj, M.S C Blagrove, L. Lytra, J. Orsborne, H. M. Al-Amin, Abdul Rahim Mohammed, M. Shafiul Alam, R. Girod, Y. A Afrane, S. Bino, V. Robert, S. Boyer, M. Baylis, E. Velo, G. L Hughes, T.Walker
   Wellcome Open Research 2024, 9:18 Last updated: 01 APR 2024
   https://doi.org/10.12688/wellcomeopenres.20761.1

6. **Review of dengue vectors in Cambodia: distribution, bionomics, vector competence, control and insecticide resistance.**
   Doeurk B, Marcombe S, Maquart PO, Boyer S.
   Parasit Vectors. 2024 Oct 9;17(1):424. doi: 10.1186/s13071-024-06481-5.

7. **Speciation patterns of Aedes mosquitoes in the Scutellaris Group: a mitochondrial perspective**
   A. Rakotonirina, C. Dauga, M. Pol, M. Hide, L. Vuth, V. Ballan, S. Kilama, S. Russet, S. Marcombe, S. Boyer & N. Pocquet
   Scientific Reports volume 14, Article number: 10930 (2024) https://doi.org/10.1038/s41598-024-61573-7

8. **In-vitro assessment of cutaneous immune responses to aedes mosquito salivary gland extract and dengue virus in Cambodian individuals**
   D. Guerrero, S. Lay, E. Piv, C. Chhin, S. Leng, R. Meng, K. E. Mam, P. Pean, A. Vantaux, S. Boyer, D. Missé, T. Cantaert
   Oxford Open Immunology, Volume 5, Issue 1, 2024, iqae003. Advance Access Publication Date: 1 April 2024 https://doi.org/10.1093/oxfimm/iqae003

3.6 Bioinformatics and Artificial Intelligence Applications Unit

The Bioinformatics and Artificial Intelligence Applications (BAIA) Unit is a newly established research unit at the Institut Pasteur du Cambodge (IPC), founded in late 2024 to advance bioinformatics and artificial intelligence applications to biomedical research. BAIA mission is to integrate research, consultancy, technical support, and training, and thus become a key driver of the use of bioinformatics and AI at IPC and beyond.

The unit is lead by Dr. Giorgio GONNELLA. He originally joined IPC in August 2023 as a Senior Bioinformatician and Computational Biologist, working jointly for the Immunology Unit and the Virology Unit. In Immunology, his primary focus was on single-cell RNA sequencing and immunoprofiling analyses. In Virology, he contributed to bioinformatics pipeline development, supporting the analysis of metagenomics and amplicon data, for public health surveillance and research. This interdisciplinary role enabled him to gain in-depth knowledge across both fields, complementing his previous extensive experience in computational biology analyses and bioinformatics tools development, employing, in the years, a plethora of technologies and programming languages.

Recognizing the growing need for bioinformatics expertise, Dr. GONNELLA conducted an institute-wide survey to assess short-, mid- and long-term bioinformatics requirements across all IPC research units. This initiative, enabled the realization of a development plan for the computational infrastructure and computational analysis needs in the next years, and laid the groundwork for the establishment of BAIA. Formally created in September 2024, the BAIA Unit became fully operational in December 2024, with the addition of two further employees, with the functions of Bioinformatics Research Engineer and Bioinformatics Systems Engineer. After an initial training period, they will assist in research and service activities of the unit.

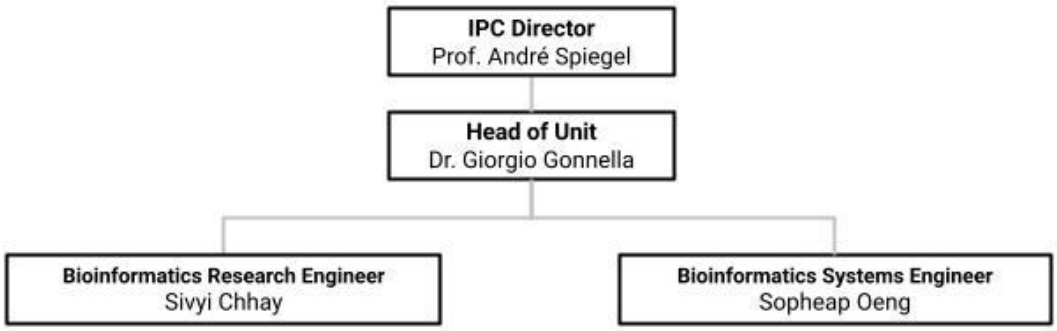
BAIA interacts with many other research units at IPC, providing bioinformatics expertise through consultancy, custom development of computational pipelines, bioinformatics data analysis of different types of sequencing data and collaborative research projects. Current internal collaborations are ongoing with the Immunology, Virology and Malaria Unit and new synergies are being explored, e.g. for microbiome analysis with the Medical Biology Laboratory. Furthermore, BAIA is actively pursuing the establishment of external research partnerships, and initiating independent projects, not solely reliant on IPC-collected data. Through its research activities, the unit aims at securing external funding to support the recruitment of further scientific and technical staff.

In addition to its research commitment, BAIA provides infrastructural support to IPC. In particular, it assists other units and IT support with advices on installation, maintenance and update of bioinformatics tools and databases. Importantly, acting as a communication interface between IPC researchers and IT support staff, it ensures finding an appropriate match between the computational needs of research and the requirements of the IT administration to ensure availability and security. To support this task, and to provide an internal communication platform, BAIA coordinates the Working Group for Bioinformatics and AI (WGBAI), with regular periodical meetings involving representatives of all research units and of the IT support. An important task of WGBAI is the coordination of the work on IPC's High Performance Computing system.

BAIA actively participates to the IPC capacity building mission by training the unit's specialized staff and offering institute-wide tutorials in IT and AI techniques, essential for advanced bioinformatics work. It plans to expand in the near future this training program to the regional Pasteur Network, organizing advanced courses available to external researchers. BAIA also welcomes intern students

from Cambodian and international educational institutions fostering knowledge exchange and capacity building.

3.6.1 Functional Structure



3.6.2 Research Programs – Major Achievements in 2024

Axis 1: Establishment of single-cell sequencing computational analysis at IPC

During the period leading up to the foundation of BAIA, and throughout its initial months, we developed and deployed computational systems tailored to the detailed analysis of immunological single cell sequencing data at IPC. These datasets, including single-cell RNA sequencing (scRNA-seq), B-cell repertoire profiling (BCR-seq) and T-cell receptor profiling (TCR-seq) are inherently complex, requiring deep expertise in both biological interpretation and in the application of advanced computational and statistical methods. In 2024, we analyzed datasets from four major projects, which will be continued in the upcoming year, investigating the immune response to infectious diseases, such as Dengue, Chikungunya and Malaria. These datasets, comprising several terabytes of sequencing data from hundreds of files, represent of an example of the complexity of this kind of analysis techniques. As a result of this work, IPC is now fully independent in performing in-house single cell sequencing data analyses, reducing the previous reliance on external collaborations for bioinformatics support, strengthening the institute's capacity for cutting-edge immunological research.

Start/End Year	2024-ongoing
Collaborations	Immunology Unit (IPC)
Funding	Institut Pasteur du Cambodge \ Immunology Unit funds and grants

Axis 2: Organization and development of computational pipelines

Computational pipelines were routinely used in the Virology Unit, before the establishment of the BAIA Unit, but many scripts and workflows were developed by non-specialized staff, often without knowledge of good engineering principles. To tackle this problem, BAIA collaborated with the Virology Unit to create new well organized and efficient computational pipelines. The first phase of this effort focused on establishing clear conventions, particularly for data storage, and restructuring existing pipelines code. Beyond virology, we developed sequencing data analysis pipelines with the Malaria Unit, applying the same principles of structured programming and reproducibility. This ongoing work ensures that IPC research units will benefit from long-term maintainability, robust reproducibility, and continuous availability of their computational workflows. The later aspect is particularly critical for

public health surveillance and outbreak response, reinforcing the IPC role in infectious disease monitoring and timely research.

Start/End Year	2024-ongoing
Collaborations	Virology Unit; Malaria Unit (IPC)
Funding	Institut Pasteur du Cambodge

3.6.3 Research Programs – Outlook 2025

Axis 1: Further development of single-cell RNA-seq computational analysis

During 2025, the Immunology Unit and its Single Cell Analysis Platform will conduct multiple new single cell sequencing studies, employing advanced techniques, such as cell hashing multiplexing and CITE-Seq. In addition to handling of new data modalities, BAIA will integrate enhanced analysis techniques in the existing established computational analysis pipelines, further refining our every aspect of it. Due to the highly heterogeneous nature of single-cell immunological datasets and of the different specific research questions associated to each project, each dataset needs to be handled with tailored downstream analyses. The expertise developed by BAIA through the analysis of previous datasets will be instrumental, ensuring comprehensive, in-depth analysis of this kind of experiments, capturing the full biological complexity of the immune system-pathogen interaction.

Start/End Year	2024-2027
Collaborations	Immunology Unit (IPC)
Funding	Institut Pasteur du Cambodge / Immunology Unit funds and grants

Axis 2: Interactive visualization and query of RNA-seq results

Tools for the interactive exploration of RNA expression across genes and cell types are important for the research community, enabling deep insights into biological processes. In collaboration with the Malaria unit at IPC, we will prepare specialized data visualization and query resources, with particular focus to the predominant species of the causative agent of Malaria in Cambodia, *Plasmodium vivax*, which presents unique biological aspects, due to its complex life cycle, including dormant liver-stage forms. This resource will designed to handle heterogeneous data, be simply extended to new datasets, once these will be available, and referencing frequently used databases, enabling effective analytical workflows. By surpassing the capabilities of existing specialized tools and generic software, it aims to offer a new important reference tool for the Malaria research community in Cambodia and beyond.

Start/End Year	2025-2026
Collaborations	Malaria Unit (IPC); A. Ruberto (Center for Tropical and Emerging Global Diseases; University of Georgia; USA)
Funding	Institut Pasteur du Cambodge

Axis 3: Expanding the use of Nanopore in research and public health surveillance

Nanopore sequencing offers significant cost advantages over other sequencing technologies. Portable Nanopore sequencers provide additional advantages, since they can be employed for pathogen real-time in-field detection and discovery. Despite these benefits, laboratory kits for library preparations and bioinformatics tools are often primarily designed for Illumina data. To address these challenges, we are actively developing software solutions that enable porting analysis pipelines to the use with Nanopore data, e.g. ensuring proper handling of the different error profile of this technology. These

new computational tools and pipelines will benefit research and public health surveillance programs in low income countries, such as Cambodia, where funding is often a limiting factor.

Start/End Year	2025-2026
Collaborations	Virology Unit; P. Cronin (Duke-NUS, Singapore); T. de Lima Campos (Fiocruz, Brasil)
Funding	Institut Pasteur du Cambodge

Axis 4: Advancing computational pipelines and automation

We will continue the development of sequencing data analysis pipelines, which we created for the Immunology and Malaria unit, and create new pipelines for new research projects of these units. A focus of BAIA is the automation and streamlining of computational workflows, reducing manual intervention and ensuring greater reproducibility of the results and stability across evolving computing environments. This includes the use of containerized solutions, which provide a stable and reproducible runtime environment, which minimizes issues related to software updates, operating system changes, and dependency conflicts. As part of this initiative, we have already created a containerized version of a virology pipeline using Docker; in future developments we will transition to Apptainer, a more secure and HPC-friendly containerization framework.

Start/End Year	2024-2027
Collaborations	Virology, Immunology and Malaria Unit (IPC)
Funding	Institut Pasteur du Cambodge

Axis 5: Applications of large language models to the scientific research at IPC

Large Language Models (LLMs) are powerful and revolutionary tools, with an enormous potential use in the context of scientific research. BAIA is exploring the applications and performance of LLMs for various research tasks. An example is the extraction of structured data from scientific literature: to achieve this, we will engineer specialized prompts, and develop benchmark datasets and a scoring system to systematically evaluate different models. Beyond literature analysis, we will experiment with and compare commonly used LLMs such as GitHub Copilot, ChatGPT, Gemini, and DeepSeek, assessing their effectiveness in programming tasks, such as the rapid prototyping of bioinformatics scripts and tool, and their suitability as bioinformatics technical support systems. To achieve this goal we use advanced features such as the customized GPT provided by ChatGPT-Plus.

Start/End Year	2025-2027
Collaborations	TBD
Funding	Institut Pasteur du Cambodge

3.6.4 Teaching and Training

One of the key goals of the BAIA Unit is capacity building. Currently, our efforts focus on training IPC scientific and technical staff in IT and bioinformatics skills. In 2024, BAIA organized its first training course on the Basics of the Linux Command Line, consisting of two sessions attended by over 20 participants. Linux is a fundamental tool in scientific computing, as many bioinformatics and computational tools rely on it. Mastering the Linux command line is essential for researchers and technical staff to fully exploit the potential of this operating system.

In the upcoming years, BAIA plans to expand its training offerings. Additional courses will include more Linux command line tutorials (Basics of Linux Command Line 2, Intermediate Linux Command Line), beginner's programming courses (Basics of Bash Scripting, Basics of Python Programming), and an

Introduction to Neural Networks and Large Language Models. This internal didactical offer has been incorporated into the Internal IPC Courses Catalog, allowing staff members to select trainings relevant to their work, as part of their annual performance evaluation process.
Furthermore, BAIA is committed to developing bioinformatics expertise in Cambodia. Currently, no bioinformatics-focused higher education programs or dedicated research groups exist in the country, except for BAIA. As a result, BAIA employees and intern students are recruited from a pool of candidates with backgrounds in either biology or IT. After joining the unit, they require extensive technical training in bioinformatics, application of AI technologies, and programming, ensuring they acquire the necessary skills to contribute effectively to the unit and the institute.

3.6.5 Outlook for upcoming 3 – 5 years

In the last decade, the rapid increase in biological data volume and complexity, driven by the advances in next generation sequencing technologies, has made bioinformatics a fundamental pillar of modern biomedical research. The BAIA Unit was established to create a strong foundation for bioinformatics development and application at IPC. Currently, most IPC research units heavily rely on international partners, with challenges related to partner availability often leading to significant delays and cross-border sharing of preliminary data. A key objective of BAIA is to reduce this IPC dependence on external collaborations for bioinformatics analyses and instead develop local in-house expertise.
The rise of Artificial Intelligence (AI) is reshaping research globally, and Cambodia is no exception. National AI initiatives have been announced for the coming years, and strategic positioning of IPC in this context is critical to maintain international competitiveness in this fast-growing field. Thus, we aim to implement techniques such as fine-tuning and transfer learning, adapting LLMs and other models to specific applications in infectious diseases research. Thereby synergies with other research units will be established: an example would include employing AlphaFold 3 for studying antibody-epitope structural interactions and epitope prediction. In addition to IPC own research, the unit will act as IPC's central interface for AI applications in biomedical research, supporting national and international collaborations in the field.
Developing countries like Cambodia face a critical shortage of bioinformatics professionals, research units, and educational frameworks in this domain. BAIA's training programs aim to develop a highly skilled workforce, with the long-term goal of creating a multiplier effect—where trained specialists will contribute to training future staff and intern students. This approach will accelerate bioinformatics capacity building in Cambodia, ensuring sustainable growth in both research and applied bioinformatics expertise.

Pasteur Asia-Pacific International Bioinformatics Initiative

Beyond IPC, BAIA aims to become an important reference point for bioinformatics research and a leading bioinformatics group in Cambodia and Southeast Asia. To foster regional collaboration, BAIA has recently proposed a Pasteur Asia-Pacific International Bioinformatics Initiative (PAPIBI). It aims at creating a bioinformatics knowledge-sharing network among Pasteur Network institutes in the Asia-Pacific region. These institute often share common goals, due to geographical proximity and similar epidemiological profiles of their host countries. Furthermore, institutes located in low-income countries often face common challenges, including limited funding, network infrastructure constraints, and hardware procurement difficulties. The initiative, set to launch in 2025, is envisioned to evolve in the following years into full-scale research collaborations, strengthening bioinformatics application and expertise in Asia-Pacific.

4 Heath Services Activities

4.1 Medical Biology Laboratory

4.1.1 Functional Structure

The Medical Biology Laboratory (MBL) provides a complete platform for biological analysis, offering around 170 tests to public and private hospitals and clinics, non-governmental organizations (NGOs), and walk-in patients. The MBL team is made up of 37 staff members working in seven divisions: Reception, Sampling, Microbiology, Mycobacteriology, Blood biology, Molecular biology, and the Bacteriology Research Laboratory (LMI DRISA / International Joint Laboratory - Drug Resistance in Southeast Asia).

In 2018, the MBL obtained ISO 15189 accreditation for biochemistry, hematology, and microbiology. In 2022, renewal of this accreditation by the French Committee for Accreditation (COFRAC) was granted for a period of 5 years. In 2024 our laboratory completed the transition to ISO 15189:2022 standard, which replaces ISO 15189:2012. The aim of this revision is to improve patient care and user satisfaction in medical laboratories through confidence in quality and competence.

In October 2024, Mr. Praveen RAHI, a researcher, joined the MBL as an International Technical Expert (ETI) from Expertise France with the mission of coordinating projects on bacteriology and antibiotics resistance within the Bacteriology and Antibiotic Resistance (BAAR) Research Group.

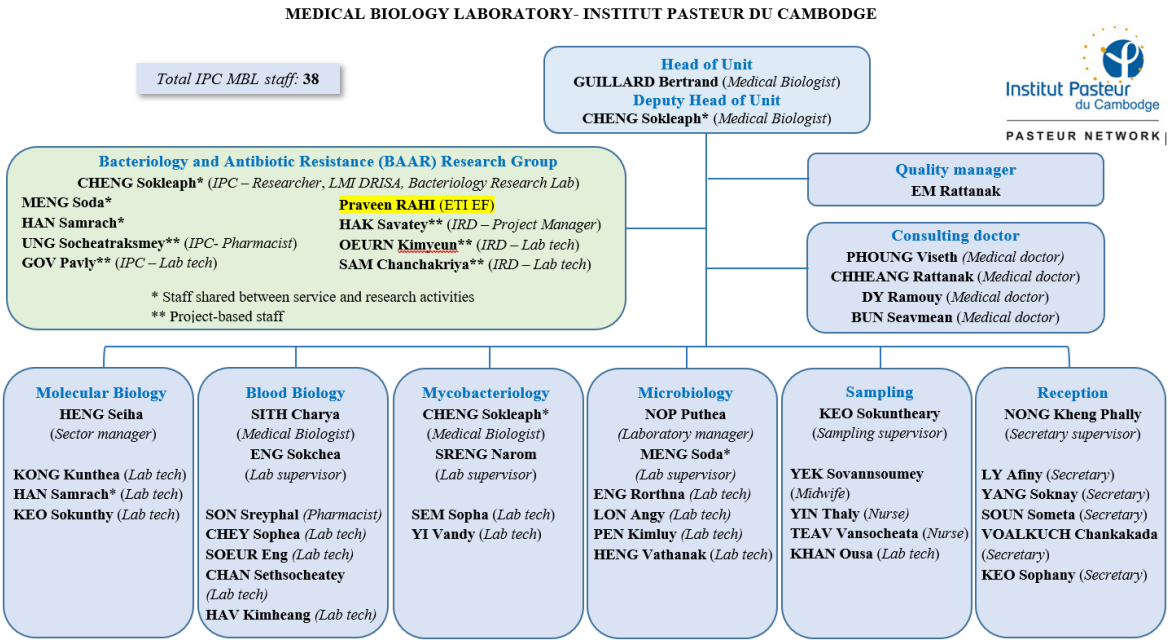


Figure 21. Organigram- Medical Biology Laboratory (January 2024)

4.1.2 Service Activities in 2024

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

In 2024, the MBL performed 163 883 analyses (-7,8% compared to 2023), resulting in 5.6 million B, a decrease of 9,2% compared to 2023.

Figure 22 illustrates the evolution of our activities over the last few years.

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

MBL sends analysis to Laboratoire Cerba in France for specialized analysis or infrequently requested tests: 272 files were sent in 2024 compared to 241 in 2023 (+ 13%).

In December 2024, MBL set up DGWeb, a web application designed to provide healthcare professionals with easy and secure access to the reports generated by our Laboratory Information System (LIS), DGLab, set up one year before.

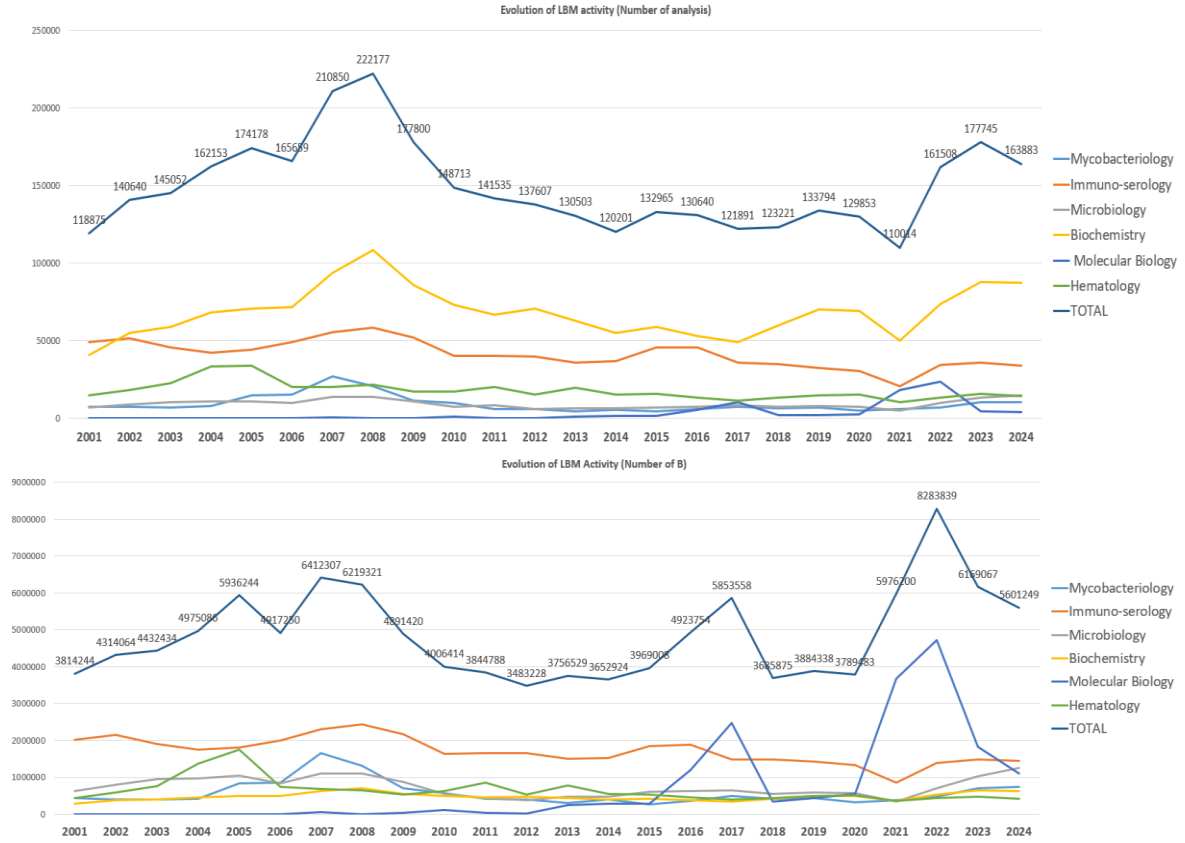


Figure 22: Trends in MBL activities, values expressed in key-letter B (adapted for the MBL using the French Nomenclature for Medical Biology Acts, or NABM)

4.1.2.1 HIV

Voluntary Confidential Counselling and Testing for HIV (VCCT)

In 2024, 259 patients consulted the VCCT and received a free HIV consultation and screening test (see Figure 23). Of these consultations, 17.8 % were HIV-positive. This high rate of positivity is due to the fact that most patients were referred by other health centres for free confirmation of a positive HIV antibody rapid test administered elsewhere. Of the 259 patients, 16 were referred by NGOs (such as Men's Health Cambodia, Reproductive Health Association of Cambodia, Chouk Sor Clinic) working with most at-risk populations, including entertainment workers, men who have sex with men and transgender people. In addition, 80 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 seen a decline in VCCT activities, due to the opening of new HIV testing

centres targeting populations most at-risk. However, the MBL's VCCT activities remain the reference for these centres in order to confirm their results.

HIV Nominative Serology (MBL routine patients)

In 2024, the number of HIV serology screenings remained the same as in 2023.

The seropositivity rate among MBL patients fell to 6.4 % (see Figure 23). This prevalence exceeds that of the general population, partly due to samples sent by other laboratories to confirm their positive results.

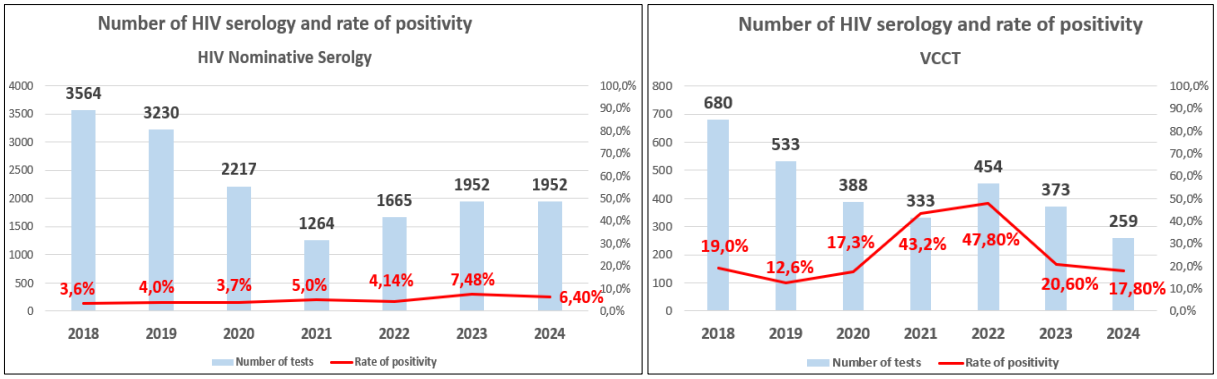


Figure 23. HIV serology and rate of positivity at VCCT and MBL

4.1.2.2. Bacteriology

In 2024, the number of bacterial cultures increased by 29%. The rate of extended-spectrum-beta-lactamase (ESBL) *Enterobacteriaceae* (27%) and carbapenem-resistant *Enterobacteriaceae* (CRE) (2%) remained stable compared with 2023. The rate of methicillin-resistant *Staphylococcus aureus* (MRSA) remains stable at 35%.

The rate of carbapenem-resistant *Acinetobacter baumannii* (CRAB) increased significantly by 15%.

14 strains of *Burkholderia pseudomallei* were isolated.

Of particular note was the isolation for the first time in our laboratory of a vancomycin-resistant *Enterococcus faecium* strain, a pathogen that has emerged worldwide as a formidable threat in healthcare settings (Table 8).

In 2024, as an alternative to the classic bacterial culture for the diagnosis of sexually transmitted infections (STIs), the MBL conducted 472 *Chlamydia trachomatis*/*Neisseria gonorrhoeae* PCRs on vaginal, urine, urethral, throat, and anal samples. Among these, 8.1 % (n = 38) tested positive for *Chlamydia trachomatis*, and 4.4 % (n = 21) for *Neisseria gonorrhoeae*, while we identified 6 co-infections (1.3 %).

Bacteria of particular public health importance	2020	2021	2022	2023	2024
Samples tor bacteriological culture	4114	2508	5023	7101	**9158**
Positive cultures with antibiogram	1466 (35,6%)	967 (38,6%)	1548 (30,8%)	2623 (36,9%)	**2994 (32,7%)**
Extended spectrum beta-lactamase (ESBL)					
Enterobacteriaceae	200/701 (28,5%)	143/503 (28,4%)	290/851 (34,1%)	405/1466 (27,6%)	**483/1769 (27,3%)**
Escherichia coli	155/366 (42,3%)	107/254 (42,1%)	240/487 (49,3%)	347/893 (38,9%)	**461/1014 (45,5%)**
Klebsiella pneumoniae	31/206 (15%)	25/127 (19,7%)	34/242 (14%)	43/341 (12,6%)	**20/455 (4,4%)**
Others	14/129 (10,9%)	11/122 (9%)	16/122 (13,1%)	15/232 (6,5%)	**2/300 (0,7%)**
Cephalosporinase hyperproduction					
Enterobacteriaceae					**53/1769 (3%)**
Escherichia coli	/	/	/	/	**37/1014 (3,6%)**
Klebsiella pneumoniae					**14/455 (3,1%)**
Others					**2/300 (0,7%)**
Third-generation cephalosporin-resistant (3GCRE)					
Enterobacteriaceae					**536/1769 (30,3%)**
Escherichia coli	/	/	/	/	**498/1014 (49,1%)**
Klebsiella pneumoniae					**34/455 (7,5%)**
Others					**4/300 (1,3%)**
Carbapenem-resistant					
Enterobacteriaceae (CRE)	26/701 (3,7%)	30/503 (6%)	40/851 (4,7%)	20/1466 (1,4%)	**34/1769 (1,9%)**
Escherichia coli	12/366 (3,3%)	21/254 (8,3%)	20/487 (4,1%)	17/893 (1,9%)	**19/1014 (1,9%)**
Klebsiella pneumoniae	9/206 4,4%)	7/127 (5,5%)	17/242 (7%)	2/341 (0,6%)	**15/455 (3,3%)**
Others	5/129 (3,9%)	2/122 (1,6%)	3/122 (2,5%)	1/232 (0,4%)	**0**
Carbapenemase types	NDM, OXA-48	NDM, OXA-48, KPC	NDM, OXA-48, KPC	NDM, OXA-48	**NDM, OXA-48**
***Acinetobacter baumannii* carbapenem-resistant (CRAB)**	9/20 (45%)	9/17 (52,9%)	8/21 (38,1%)	21/78 (26,9%)	**41/98 (41,8%)**
***Salmonella typhi* fluoroquinolone-resistant**	/	/	/	/	**1/4 (25%)**
***Enterococcus faecium* vancomycin-resistant**	0/12	0/6	0/25	0/15	**1/29 (3,4%)**
***Pseudomonas aeruginosa* carbapenem-resistant (CRPA)**	/	/	/	/	**5/163 (3,1%)**
***Salmonella non-typhi* fluoroquinolone-resistant**	/	/	/	/	**2/8 (25%)**
Neisseria gonorrhoeae					
third-generation cephalosporin-resistant	0/11	3/6 (50%)	1/4 (25%)	1/1 (100%)	**1/2 (50%)**
fluoroquinolone-resistant	/	/	/	/	**2/2 (100%)**
***Staphylococcus aureus* methicillin-resistant (MRSA)**	93/238 (39,1%)	43/139 (30,9%)	71/204 (34,8%)	116/333 (34,8%)	**135/391 (34,5%)**
Salmonella paratyphi A	37	10	2	1	**8**
Burkholderia pseudomallei	14	22	15	18	**14**

Table 8. Bacteriological data in 2024

4.1.2.3. Tuberculosis

In 2024, 10326 analyses were carried out for service activities, almost identical to the previous year. In 2024, 2030 Xpert MTB/RIF Ultra tests were performed for rapid TB diagnosis, of which 20.7% were positive for *Mycobacterium tuberculosis* complex (MTBC) and 1.2% of positive samples showed resistance to rifampicin.

Figure 24 illustrates the evolution of rifampicin resistance in *M.tuberculosis* over the last 6 years.

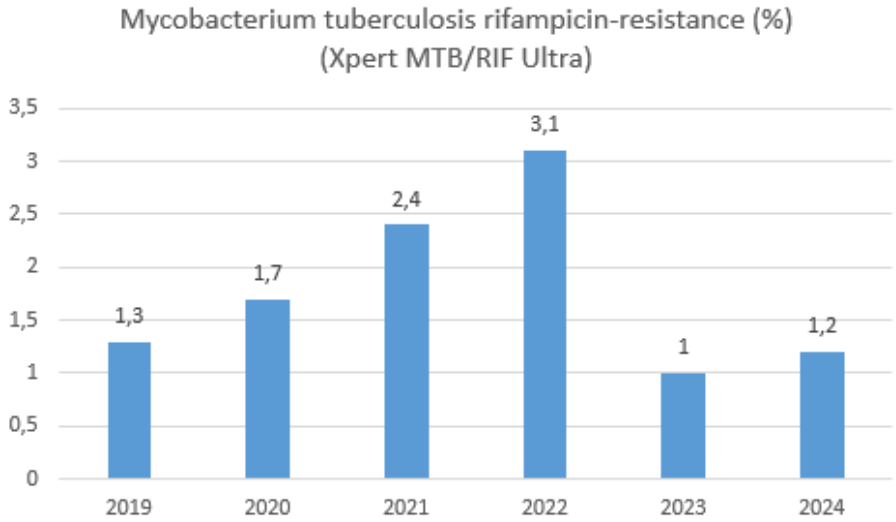


Figure 24. Evolution of rifampicin resistance in M.tuberculosis 2019-2024 (Xpert MTB/RIF)

In addition to the service activities, the Mycobacteriology laboratory also participated in the 3rd National TB prevalence survey as a culture-performing laboratory for the survey. The survey ended in May 2024, with 460 sputum samples received in 2024 for smear microscopy and culture.

4.1.3 Research Programs - Major Achievements in 2024

CircUs - Pilot phase in Cambodia

This multicenter study aims to investigate the circulation of Antimicrobial Resistance (AMR), particularly focusing on Multi-Drug-Resistant Enterobacteriaceae (MDR-E) and mobile genetic elements across humans, animals, and the environment in Burkina Faso, Cambodia, Ivory Coast, and Madagascar. Takeo Provincial Referral Hospital was selected as a study recruitment site. At present, data collection from the hospital work package (WP1) and the patient household and environment work package (WP2) has been completed. 64 patients were recruited in WP1.

37 (57.8%) patients out of 64 had MDR-E: 27/37 (42.2%) had ESBL, 4/37 (6.3%) had CPE (carbapenemase-producing Enterobacteriaceae) and 6/37 (9.4%) had both ESBL and CPE. In WP2, 36 patient households were visited and 406 samples were collected (human, animal, rodent, environmental and food samples). Of these 406 samples, 357 (87.9%) had MDR-E. Some strains will be selected for gDNA analysis and next-generation sequencing at the Institut Pasteur de Paris for WP3. CircUs-Cambodia's aim is to complete the results and final report by 2025.

Collaborations	PIs: N. GUESSEND (IP in Ivory Coast), A. SALAM, E. CARDINAL (Cirad), A.L. BAÑULS (IRD) Team Leader for Cambodia: S. CHENG (MBL) MBL: B. GUILLARD, V. HENG LMI-DRISA$_{OH}$: P. GAO, S. HAK Takeo Provincial Referral Hospital: S. SEANG General Directorate of Animal Health and Production (GDAPH), MAFF: S. SAN
Funding	AVIESAN AMR-SUD ANR “Antibiorésistance : comprendre, innover, agir (AMR)"

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

Nerve angiostrongylosis is an anthropozoonosis caused by *Angiostrongylus cantonensis*, a parasitic nematode. The parasite cycle of *A.cantonensis* requires the presence of definitive hosts (mainly rats) and intermediate hosts (snails, notably *Achatina fulica*) for its complete maturation. Humans are incidental hosts and infection can occur through the consumption of raw or undercooked intermediate or paratenic hosts. Neurologic manifestations include eosinophilic meningitis, encephalitis/encephalo-myelitis. Today, human cases of nerve angiostrongylosis are primarily described in Southeast Asia (Thailand, Malaysia, China), the Pacific Islands, Indian Ocean islands, the Greater Antilles (Cuba, Puerto Rico, Jamaica), and more recently Brazil. The ORACAN project, funded by the Institut Pasteur through Actions Concertées InterPasteuriennes - ACIP 2022, aims to determine the origin of the *A.cantonensis* and introduction pathways (rats or snails). This involves assessing prevalence and generating phylogenetic data of *A.cantonensis* and its major intermediate host, *A.fulica* in all regions of the world. In 2024, the activities included the collection of snails, collection of rodents tissue sample from the IPC Biobank, and laboratory testing. Out of 140 rodent samples

provided by the MBL and Virology Unit in IPC, 5 samples were positive for A. cantonensis. All *Achatina fulica* collected were negative for *A. cantonensis.* Some rodent DNA barcoding remains to be done. In 2025 the submission of regional publications is planned, then will be submitted the review publication.

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

Households and close contact investigations of persons with tuberculosis in Cambodia

Cambodia, having recently transitioned out of the global list of high TB-burden countries, still faces challenges in identifying and treating TB cases effectively. Current national guidelines and World Health Organization (WHO) recommendations for TB contact investigations focus on bacteriologically confirmed cases, excluding individuals with smear-negative TB who could still transmit the infection. Additionally, a significant proportion of TB cases in Cambodia are detected without the typical symptoms of coughing. Despite this, the prevalent strategy relies heavily on symptom screening, potentially missing a substantial number of TB cases. Notably, testing for TB infection (TBI) using tuberculin skin tests (TST) or interferon-gamma release assays (IGRA) is not routinely implemented in Cambodia. The project aims to fill this gap by utilizing IGRA to assess the feasibility of screening latent TB infection rates among household and close community contacts with bacteriologically and non-bacteriologically confirmed pulmonary TB (regardless of symptoms) and identify factors associated with TB infection among eligible household contacts. The MBL collaboration in the project ensures the proper performance of the IGRA test as per protocol and provides timely results to decide whether to initiate preventive therapy. Patient recruitment and IGRA testing started in October 2023 and finished in August 2024. Analysis of 250 people recruited in urban area (Prek Pnov, Phnom Penh) revealed that 25% of them were IGRA positive. Of these, 90% were contacts of bacteriologically confirmed TB cases, and 10% were contacts of clinically diagnosed TB. In 2025, a second phase of the project is planned with larger and more representative sampling.

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

PREZODE AfriCam Cambodia

As part of the fight against zoonotic diseases and the international PREZODE (Preventing Zoonotic Disease Emergence) initiative, the AfriCam Cambodia project, funded by the French Development Agency (AFD), was launched on June 1st, 2023, in Phnom Penh. The aim of AfriCam Cambodia is to study zoonotic risks associated with hydrological dynamics and climatic and environmental changes at the human-animal-environment interface. The project will implement activities aiming to reduce the emergence of zoonotic risks and strengthen the surveillance system towards "One Health" surveillance. The MBL is involved in activities such as (1) assessing the circulation/exposure of zoonotic pathogens (bacteria and parasites) in humans, animals, and the environment, (2) investigating the etiology of unexplained syndromes (bacterial infections), and (3) implementing tools for the early detection of infectious risks (e-DNA). In 2024, the LBM focused on obtaining the support of partners and local authorities at the study sites. Some technical activities, including the development of study protocols, staff training, optimization of methods and submission of studies (serological survey, Battambang epidemiological study and KAP survey) to the ethics committee, have also progressed. AfriCam-MBL aims to begin its field mission in March 2025 and to complete its field activities in June 2026.

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 (Powering entrepreneurs to end poverty), WCS (Wildlife Conservation Society), Battambang Hospital, Institute of Technology in Cambodia (ITC), WCS (Wildlife Conservation Society)
Funding	AFD

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

Nontuberculous mycobacteria (NTM), known to cause chronic infections, are environmental pathogens commonly found in water and soil. The incidence of NTM infections is increasing dramatically in Australia, Japan, the United States, and other global regions. In many Asian countries, however, epidemiological data on NTM are lacking as public health prioritizes tuberculosis cases. Therefore, this multi-party collaboration will evaluate the epidemiology of NTM infection in Australia, the US, Japan, Thailand, and Cambodia. We will document the impact of the dispersal of environmental NTM that occurs following major weather events through geospatial analysis of infection incidence, in parallel with climate and weather data, and use modeling techniques to forecast future disease patterns. The specific aims of this study are: (1) To identify climate change variables and major weather events that predict patterns of NTM incidence in Australia, the US, Japan, Thailand, and Cambodia; and (2) To evaluate novel disinfection methods that can be applied to drinking water distribution systems to mitigate the impact of NTM dissemination caused by major weather events and climate change (Australia only). Following the Kick-off meeting in April 2024, the study protocol was submitted and approved by the National Ethical Committee for Health Research in September 2024. In 2024, NTM database summarizing laboratory's information has been created. The project activities in 2025 will focus on compiling a comprehensive report on NTM epidemiology and climate data covering the period from January 2010 to April 2024 in Cambodia.

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

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

Histoplasmosis, talaromycosis, and cryptococcosis are serious invasive fungal infections (IFI) in patients with advanced HIV disease. Even though these pathogens are probably endemic in Cambodia, little or no data are available, especially for histoplasmosis and talaromycosis. The lack of awareness of these infections and the insufficient availability of reliable diagnostic methods lead to delay diagnosis, resulting in a late introduction of specific treatment and an excessive burden. In recent years, antigenic and molecular techniques have emerged as promising tools for the rapid diagnosis of IFI. Our main objective is to assess, using these new methods, the prevalence of these IFI in Cambodia in patients living with HIV at an advanced stage (CD4 T lymphocytes <100/mm3) with no previous antiretroviral therapy (ART) and having participated in the STATIS study (ANRS_12290), a study on the management of tuberculosis associated with HIV. Our secondary objective is to raise awareness and train local actors on these infections with new and simple diagnostic tools. The project received funding in 2023 from ANRS MIE, and the study protocol was approved by Cambodia's National Ethical Committee for Health Research in February 2024. A four-day Laboratory Training Workshop took place in October 2024 at the MBL, covering both theoretical and practical aspects of diagnosing these invasive fungal infections.

Collaborations	PIs: S. Cheng (Institut Pasteur du Cambodge, Cambodia) and A. STURNY LECLERE (Institut Pasteur de Paris, France) E. MOSNIER, University of Health Sciences, Cambodia A. ADENIS, Cayenne Hospital, French Guiana, France N. De Rekeneire, Institut Pasteur du Cambodge, Cambodia
Funding	Inserm - ANRS MIE Sponsor: IPC

LMI-DRISA$_{OH}$ "Laboratoire Mixte International - Drug Resistance in Southeast Asia: a one Health approach to tackle AMR spread"

This is a continuing project from LMI-DRISA that was established in 2016 as part of a regional effort involving Vietnam, Cambodia, and Lao PDR. The main objective of the LMI is to share competencies and best practices among different academic and non-academic institutions in the three mentioned countries and France to study the mechanisms and factors influencing the emergence and transmission of drug resistance and their implications for public health in Southeast Asia. To address this complex situation, the second phase of this 5-year project (2021-2025), named 'LMI-DRISA$_{OH}$,' aims to integrate a 'One Health' approach to understand antimicrobial resistance emergence and spread at the interface of humans, animals, and the environment. In 2024, the LMI-DRISA$_{OH}$ activities in Cambodia focused on supporting ongoing project activities (CircUs and AfriCam), data analysis and valorization (FSPI ARCAHE and FSPI Wat-Health) and capacity building (support MBL research in

pathogens and resistance determinants through serological and molecular detection techniques, ongoing evaluation of the possible implementation in MBL of the microscopic agglutination test (MAT) for the serology of leptospirosis.

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 (B. GUILLARD) Centre d'Infectiologie Lao Christophe Mérieux (CILM) Fondation Mérieux, National Institute of Hygiene and Epidemiology (NIHE) University of Science and Technology of Hanoi (USTH) Oxford University Clinical research (OUCRU)
Funding	IRD (2020-2025)

4.1.4 Research Programs - Outlook for 2025

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

Cervical cancer screening is a global and Cambodian public health priority, with emerging methods indicating superiority over existing VIA-based screening. The WHO recommends primary HPV screening as the preferred strategy wherever affordable. Important country-specific implementation issues include the choice of the most effective triage test for HPV-positive women and the most accepted mode of HPV test collection. In 2023 the MBL carried out High-risk HPV (hrHPV) testing using the Cobas4800® platform (Roche): 10,075 samples were tested (detection of HPV16, HPV18 and other 12 pooled hrHPV genotypes). The analysis of the data is ongoing. In 2025 additional tests will be performed on the HPV-positive samples: identification of the hrHPV genotypes individually and methylation assay (which could help for the triage of advanced cervical intraepithelial neoplasia (CIN) among hrHPV-positive women).

Collaborations	PIs: H. BUSSMANN (Heidelberg University) and Dr S. VONG (Ministry of Health) MBL: S. CHENG, S. HENG, B. GUILLARD
Funding	Heidelberg University, Oncgnostics GmbH

Diagnostics and surveillance of acute meningo-encephalitis among children in Cambodia with a focus on Japanese Encephalitis Virus (DEMELE-JEV project)

Infectious meningitis and encephalitis are significant public health concerns in Southeast Asia due to their potential for rapid epidemic spread and high morbidity and mortality. These conditions are primarily caused by viruses and bacteria, with Japanese encephalitis virus (JEV) being the leading cause of viral encephalitis in Asia. Despite the introduction of the JEV vaccine in Cambodia's national immunization program in 2015, the disease remains prevalent, especially among children. This situation is exacerbated by diagnostic limitations and insufficient surveillance. Recent advancements in diagnostic technologies, such as multiplex molecular platforms, offer new opportunities to improve the detection and management of these diseases in resource-limited settings like Cambodia. Objectives are to quantify the clinical burden of Japanese Encephalitis (JE) and the asymptomatic circulation of JEV among Cambodian children, to estimate anti-JEV seropositivity rates, to identify the

etiologies of febrile neurological syndrome in children, to develop and evaluate new tools for diagnosis. Children will be recruited in Kantha Bopha hospitals in Phnom Penh and Siem Reap. The MBL will perform TB testing (GeneXpert, culture). Project submitted to Cambodia's National Ethical Committee for Health Research; activities should start in April 2025.

Collaborations	PIs: Pr SAPHONN Vonthanak (University of Health Sciences, Cambodia), Dr DUBOT-PERES Audrey (Unité des Virus Émergents, Aix-Marseille Université, France)
Funding	MEAE / ANRS-MIE (2024-2026)

Households and close contact investigations of persons with tuberculosis in Cambodia - Phase 2

"Households and Close Contact Investigations of Persons with Tuberculosis in Cambodia-Phase 2" study is set to begin in 2025, following the completion of Phase 1 in August 2024. Phase 1 aimed to assess the feasibility of using the interferon-γ release assay (IGRA) to screen for TB infection and determine its prevalence among households and close community contacts in urban areas in Cambodia. However, the results were limited due to the small sample size, necessitating further verification with larger, more representative samples. To address this, Phase 2 will expand the study to include rural areas (in Kampot, Prey Veng and Ratanakiri provinces), improving geographic representation. The study protocol was submitted and approved by Cambodia's National Ethical Committee for Health Research in August 2024. In 2025, the phase 2 of the project will involve 500 participants from rural areas. The results of this phase will guide the implementation of household-based TB contact tracing and testing for TB infection, providing essential data for policymaking in Cambodia.

Collaborations	PIs: C. HOUT (National TB Control Program) MBL: B. GUILLARD, S. CHENG, S. UNG University of Sydney, Australia: AKJ. TEO KHANA, Cambodia: S. TUOT
Funding	United States Agency for International Development (USAID) Dynamic Pharma Co., Ltd provides the equipment to be used to perform the ELISA test for the duration of the study.

ExposUM PEACH: Pathogens Exposure from Aquifers: A Cambodian Health interdisciplinary case study

In Cambodia, the upper Mekong delta is subject to large natural flooding every monsoon season. Climate change, dam building upstream, irrigation and drainage infrastructures modify the flood dynamics, resulting in shorter flooding periods and shallower water levels. These modifications in water conditions have been directly associated with the presence of waterborne pathogens like *Burkholderia pseudomallei* and *Leptospira interrogans*, responsible for melioidosis and leptospirosis, respectively. The study entitled Pathogens Exposure from Aquifers: A Cambodian Health interdisciplinary case study (PEACH) proposes to characterize the role of groundwater in the environmental exposure to bacteria in the floodplain of the upper delta of the Mekong River. The study aims to gain essential knowledge on the ecology of *L. interrogans* and *B. pseudomallei* and define how shifting hydric conditions influence the origin and timing of the associated water exposome, impacting public health. In 2024, the MBL activities mainly focused in study development and field sampling plan. To date, one of the missions of soils sampling and water vertical sampling was

completed to integrate bacterial spatio-temporal distribution, hydrodynamic drivers and evaluation of population vulnerability to water-related hazards. In 2025 further missions are planned.

Collaborations	PI: S. MASSUEL (IRD) IPE: M. HIDE (IRD) MBL: S. CHENG, and P. GAO IRD: S. HAK, and C. SAM
Funding	Montpellier University

4.1.5 Support to National Authorities

The Mycobacteriology laboratory took part in the 3rd national TB prevalence survey (460 sputum samples received in 2024 for smear microscopy and culture).

After being informed by the Cambodian CDC of cases of anthrax in Laos in March, the MBL set up a molecular identification technique for *Bacillus anthracis*. The MBL was not subsequently asked to carry out analyses.

As part of its training mission, the MBL cooperated at the request of the Ministry of Health (Cambodia) for the training of human resources:

- NCHADS Laboratory staff strengthening:
  Currently, The National Center for HIV/AIDS, Dermatology and STD (NCHADS) outsources HIV Drug Resistance (HIVDR) genotyping testing to the MBL of IPC. In order to be able to perform HIVDR genotyping tests in its own laboratory in the future, the MBL trained a member of NCHADS staff for 3 months in 2024. On this occasion a medical laboratory services agreement was signed between IPC and NCHADS.
- Provincial Hospital Microbiology Laboratory staff strengthening:
  At the request of the National Medical Microbiology Laboratory Network (MoH) one member of staff from the Svay Rieng Hospital Laboratory and one from the Bantheay Meanchay Hospital Laboratory are being trained in the Medical Microbiology laboratory of the MBL for a six-month period (December 2024 to May 2025) in order to enhance their capacity.

4.1.6 Teaching and Training

Continuing Professional Training and Development for the MBL's Staff in 2024

Date	Training topic	Trained by	Staff trained
2024	PCR ADNr 16S universelle au service du diagnostic en Bactériologie ; Arboviroses; Leptospirosis ; Maladies d'origine Génétique ; Le syndrome de Cushing ; La maladie d'Alzheimer du point de vue de la biologie ; L'hyperlymphocytose : intérêt de la cytométrie en flux ; Décryptage de la maladie cœliaque : pour un diagnostic efficace ; Syndrome des anti phospholipides ; Place et intérêt de la cytogénétique dans la prise en charge des hémopathies malignes	CERBA Live Session	SITH.C, HENG.S, NOP.P, PHOUNG.V, B.GUILLARD, CHHEANG.R

2024	General English Program	Australian Center for Education (ACE)	CHOR.L, SITH.C, NOP.P, ENG.S, CHAN. S, KHAN.O, SEM.S, HAN.S, SRENG.N, YI.V
Jan 2024	Biological & chemical risks	Kaptitude	LON.A, SIM.S, PEN.K
24/01/2024	Improving Laboratory Compliance: Strategies to address deficiencies	College of American Pathologists (CAP)	CHOR.L
02-03/02/2024	Fire Prevention and Firefighting	IPC	SIM.S
28/02/2024	ISO15189:2022 Certified Auditor Training	PUNYAM ACADEMY	ENG.S, CHENG.S, SITH.C
01/03/2024	Customer training of QuantiFERON®-TB Gold Plus ELISA (08/08/2023-09/08/2023)	QIAGEN	ENG.S, UNG.S
19/03/2024	Training for IPC Biologist on DGLab	Datamed	B.GUILLARD, CHENG.S, NOP.P
19/03/2024	Training for IPC lab supervisors on DGLab	Datamed	ENG.S, SRENG.N
19/03/2024	Training for IPC Technicians on DGLab	Datamed	All Technicians
19/03/2024	Training for IPC Secretaries	Datamed	All Secretaries
30/04/2024-07/05/2024	High Pure Extraction and Light Cycler 480 Operator Training (Bacillus anthracis Light Mix Workflow)	Roche	CHENG.S, SITH.C, HENG.S, NOP.P, KEO.S, KONG.K, HAN.S
06-10/05/2024	Scientific Writing Course	Epiconcept	CHENG.S, UNG.S
23-24/05/2024	BSL3 Emergency Response Drills	IPC	SRENG.N, SEM.S, YI.V
20/06/2024	“Management of Acute Asthma exacerbation in children”; “The Management of COPD with NEW update 2024”	Webinar-Boehringer Ingelheim / DKSH	CHHEANG.R
03/08/2024	Attended annual scientific meeting	Cambodian Heart Association	CHHEANG.R
10/08/2024	“Advanced Digestive Endoscopy & Helicobacter pylori Eradication”	GAGE	CHHEANG.R
29/08/2024	Cardiopulmonary resuscitation (CPR)	MC&Co Prevention	YEK.S, HENG.S, KEO.S
12-13/09/2024	Excellence in Customer Service: Making the Sale	EuroCham Cambodia	NONG.KP

10-11 /10/2024	cobas® HBV for use on cobas ® 4800 training course	Roche	HENG.S, HAN.S, KEO.S, KONG.K, NOP.P, SITH.C, B.GUILLARD

Internships

The medical biology laboratory (MBL) has played a crucial role in training. In 2024, MBL welcomed 96 university students, civil servants and high school students who took part in its training program to improve their skills and knowledge.

- University of Health Science (UHS): 75 students (Pharmacists, technicians, biologists)
- University of Puthisastra (UP): 12 students (Pharmacists)
- Lycée Français René Descartes (LFRD): 6 High school students
- Cambodia Japan Friendship Mongkul Borey Referral Hospital (CJFMBRH): 1 Civil servant
- Svay Rieng Provincial Referral Hospital (SRPRH): 1 Civil servant
- NCHADS: 1 Civil servant

Table 9: Description of trainees in Medical Biology Laboratory:

Trainee	Program	Institute	Start date	End date	Trained in
Civil servant	In service	CJFMBRH	02-12-2024	30-05-2025	Microbiology
Civil servant	In service	SRPRH	02-12-2024	30-05-2025	Microbiology
6 students	ADL lab-year 3	UHS	25-12-2023	27-01-2024	Lab technique
5 students	BA lab-year 2	UHS	29-04-2024	30-04-2024	Quality
Civil servant	In service	NCHADS	02-05-2024	02-08-2024	Molecular biology
5 students	BA lab-year 2	UHS	06-05/2024	12-06-2024	Lab technique
5 students	BA lab-year 2	UHS	13-06/2024	18-07-2024	Lab technique
1 student	High school	LFRD	19-06-2024		Lab technique
8 students	BA Pha-year 4	UHS	01-07-2024	31-07-2024	Lab technique
8 students	BA Pha-year 4	UHS	05-08-2024	13-09-2024	Lab technique
9 students	BA Pha-year 4	UHS	16-09-2024	25-10-2024	Lab technique
8 students	BA Pha-year 4	UHS	28-10-2024	19-11-2024	Lab technique
8 students	ADL lab-year 3	UP	12-08-2024	26-10-2024	Lab technique
16 students	BA nurse	UHS	05-09-2024		Observation
4 students	MMB-year 2	UHS	18-11-2024	14-02-2024	Lab technique
4 students	BA res- year 2	UP	07-10-2024	01-11-2024	Microbiology
1 student	BA lab- year 2	UHS	18-11-2024	23-11-2024	LQMS
5 students	High school	LFRD	16-12-2024	20-12-2024	Lab technique

4.1.7 Outlook for 2025

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 maintain our level of technical expertise. In December 2025, a surveillance audit will be carried out by the *French Committee for Accreditation* (COFRAC).

Development plan

The MBL of IPC must adapt to the changing landscape of medical biology in Cambodia. In 2025, a development plan for the laboratory will be put in place. This plan involves collaboration between MBL and the IPC management:

- Opening of the laboratory on Saturday afternoons
- Extension of opening hours
- Revision of the prices of analyses in the MBL catalogue
- Introduction of new tests and acquisition of new equipment
- Consideration of setting-up an off-campus sampling site

Research activities

- Strengthening research capacity: The creation of the BAAR group in 2022 was a significant step forward. However, the group currently consists of 5 five people and a single researcher, who can only devote 50% of her time to research activities. It is therefore imperative to strengthen the team's research capacity by recruiting PhD students, post-docs, or visiting scientists as part of research collaborations.
- Strengthen expertise in statistical data analysis, modern clinical microbiology, genomic, and bioinformatics.
- Increase the number of scientific publications: it is essential for the team to publish the data generated by various projects, which has been delayed due to routine activities and limited human resources.

4.1.8 Scientific Publications 2024

Note: *The name of authors from the Institut Pasteur du Cambodge are underlined*

1. **Antibiotic resistance profiles of sentinel bacteria isolated from aquaculture in Cambodia.**
   Peng C, Moniroth S, Khy P, Chea S, Thanh C, Heng O, Sarter S, Cheng S, Caruso D.
   J Water Health. 2024 Jun;22(6):1033-1043. doi: 10.2166/wh.2024.101. Epub 2024 May 8.
   PMID: 38935454, https://doi.org/10.2166/wh.2024.101

2. **Bayesian accuracy estimates for diagnostic tests to detect tuberculosis in captive sun bears (Helarctos malayanus) and Asiatic black bears (Ursus thibetanus) in Cambodia and Vietnam.**
   Officer K, Arango-Sabogal JC, Dufour S, Lyashchenko KP, Cracknell J, Thomson S, Cheng S, Warren K, Jackson B. PLoS One. 2024 Nov 13;19(11):e0313007. doi: 10.1371/journal.pone.0313007. eCollection 2024.

3. **Colistin resistance in ESBL- and Carbapenemase-Producing Escherichia coli and Klebsiella pneumoniae clinical isolates in Cambodia.**
   Hide M, Meng S, Cheng S, Bañuls AL, Ky S, Chantana Y, Laurent D, Delvallez G.
   J Glob Antimicrob Resist. 2024 Jul 12:S2213-7165(24)00134-6. doi: 10.1016/j.jgar.2024.06.017. Online ahead of print. PMID: 39004342

4. **Genomic insights into anthropozoonotic tuberculosis in captive sun bears (Helarctos malayanus) and an Asiatic black bear (Ursus thibetanus) in Cambodia.**
   Officer K, Walker TM, Cheng S, Heng S, Hidé M, Bañuls AL, Cracknell J, Broadis N, Thy N, Abraham S, Warren K, Jackson B.

Sci Rep. 2024 Mar 28;14(1):7343. doi: 10.1038/s41598-024-57318-1. PMID: 38538629

5. **Multilocus sequence typing of clinical *Burkholderia pseudomallei* isolates from Cambodia.**
Gyamfi E, Delvallez G, Cheng S, Meng S, Oeurn K, Sam C, Kerleguer A, Guillard B, Bañuls AL, Hide M. - PLoS Negl Trop Dis. 2024 Nov 14;18(11):e0012652. doi: 10.1371/journal.pntd.0012652.

4.2 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.

4.2.1 Functional Structure

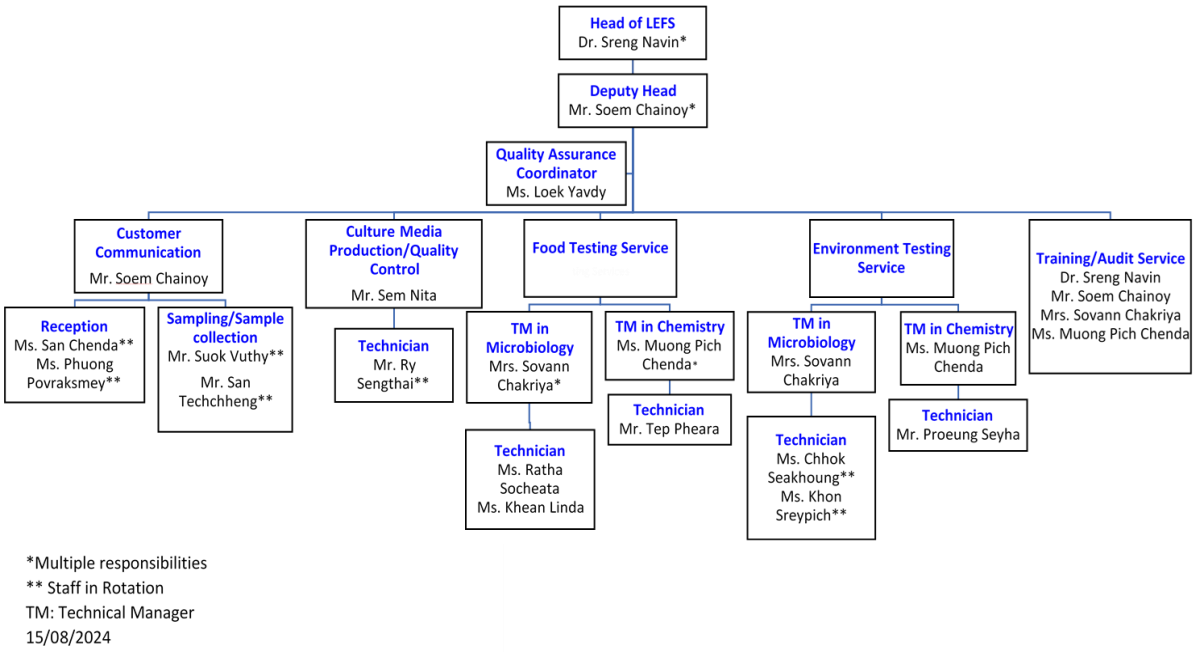


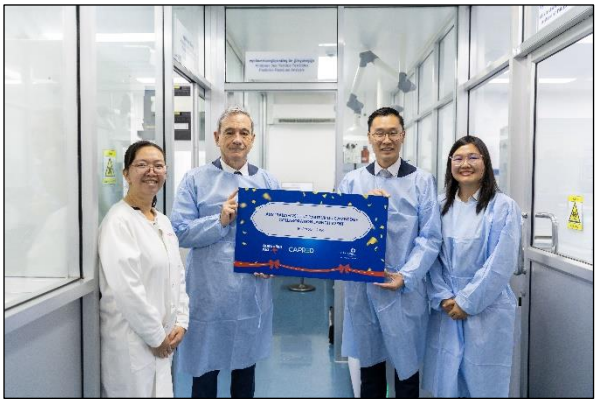
Figure 25. Laboratory of Environment and Food Safety organogram

The team's composition changed in 2024 with the recruitment of a new technician to support expanded activities in chemistry. That same year, we received financial support from the Cambodia Australia Partnership for Resilient Economic Development (CAPRED) to establish new services for heavy metal testing, as well as pesticide and antibiotic residue testing.

CAPRED program:

In September 2023, our laboratory of Environment and Food Safety has welcomed the CAPRED team and their expert for a brief laboratory assessment and visit. Following that meeting, we shared our business plan for testing heavy metals, pesticides, and antibiotic residues. Since then, we have worked together on exploring investment possibilities. After completing a market survey, we officially signed our collaboration agreement in April 2024.

On 16th October 2024, LEFS/IPC and Australia jointly organized "Australia-Institut Pasteur du Cambodge collaboration Launch event" presided by Professor André Spiegel, director of IPC, and H.E. Derek Yip, Australian Ambassador to Cambodia. There were 40 participants (female: 19) who were from DFAT, Public sector, Development Partners, domestic and international business associations, private companies. Key objective was to establish accredited laboratory services with ISO/IEC 17025:2017 for **Heavy Metal**, **Pesticide and Antibiotic testing** to promote food safety and export. It is a 2.5-year project from April 2024 to Dec 2026 (https://www.youtube.com/watch?v=8GV6851-8ks&t=2s).



Heavy Metal Lab

- The Graphite Furnace Atomic Absorption Spectrometry (GFAAS)was completely set up by the end of March 2024.
- Staff training on machine operation and test method analysis was conducted from April 2024 to the end of July 2024.
- The service became available to the public on 19th August 2024.
- A total of 193 tests were conducted from the opening of the service until 31st December 2024.

Antibiotic Residue Lab

- The equipment was installed in September 2024.
- Staff training on machine operation and test method analysis was also conducted in September 2024.
- The service is planned to be available to the public in 2025.

Pesticide Residue Lab

- The equipment was installed in September 2024.
- Staff training on machine operation and test method analysis was conducted from September to November 2024
- Method validation was performed for approximately 400 compounds in November 2024.
- The service is planned to be available to the public in March 2025.

4.2.2 Service Activities in 2024

In 2024, our laboratory analyzed a total of 8,476 samples, including 2,038 food samples, 3,043 water samples, 1,258 surface and air samples for microbiological testing, and 68 food samples along with 2,069 water samples for chemical testing. Additionally, through our partnership with b.Consulting on food safety training, we successfully conducted seven training sessions.

Compared to 2023, the total number of samples tested increased by 8.5%, while the overall number of tests rose by 4.4%.

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

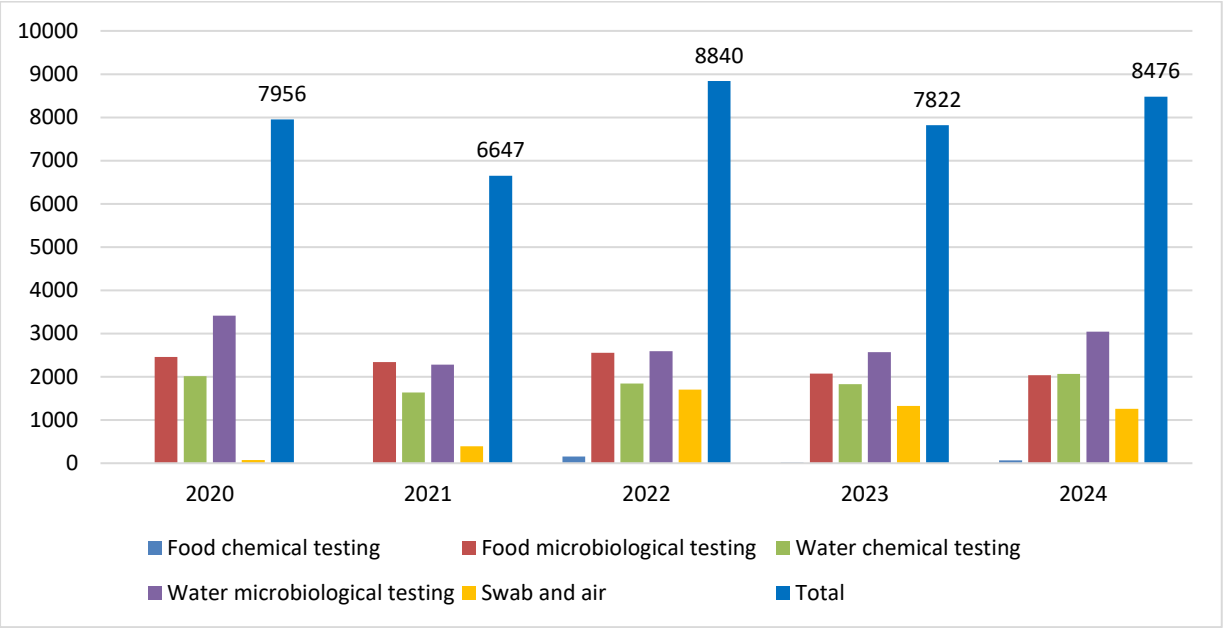


Figure 26: Evolution of the number of samples by year (2020-2024)

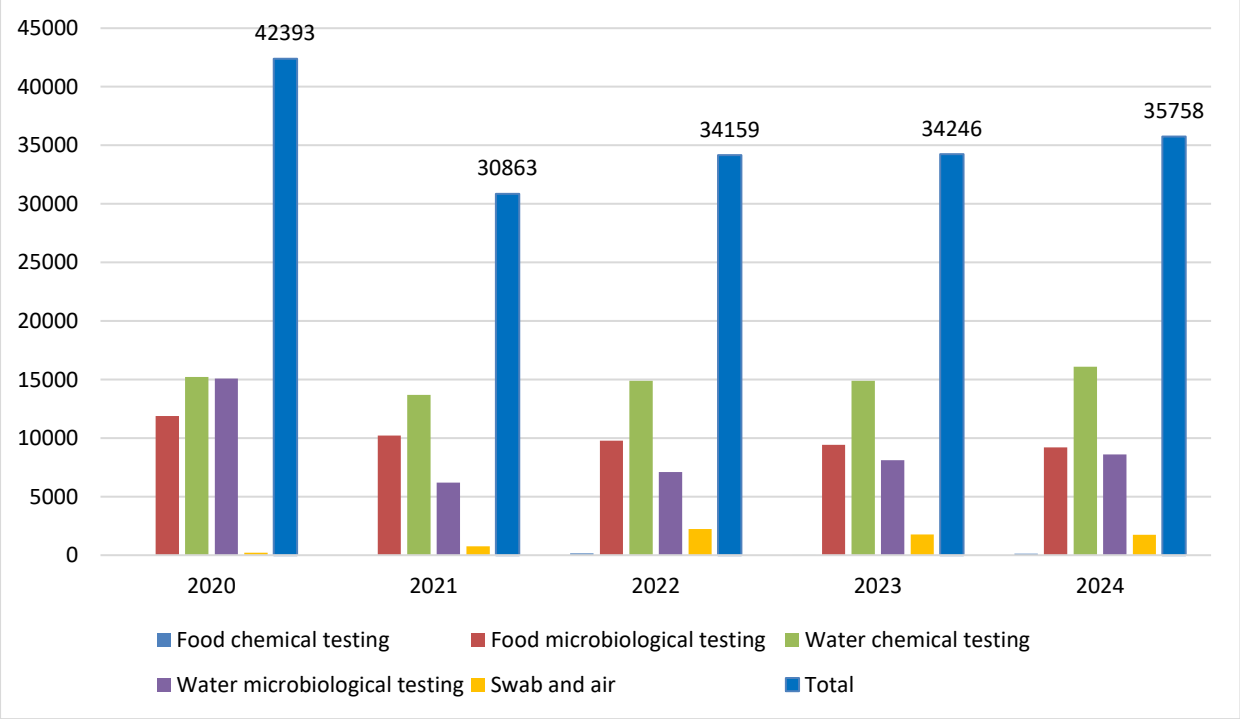


Figure 27: Evolution of the number of analysis by year (2020-2024)

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

Food sample

- 27 % of food microbiology samples (559/2,038) were requested to compare the results with reference standards or customer requirements, and 24 % (134/559) were found to be unsatisfactory.
- The unsatisfactory results were due to *Salmonella* contamination (20%), and to high levels of hygiene risk indicators such as coliform bacteria (24%), *E. coli* (19%), *Bacillus cereus* (10%), Total plate count (19%), Clostridium perfringens (5%), and yeast and mould (3%).
- 55.6 % of Salmonella-positive food samples were raw meat (15/27) followed by salad at 29.6 % (8/27), and fish and fishery products at 14.8 % (4/27).

Water

- 30 % of water samples sent to microbiology testing (922/3,043) were requested to compare the results with reference standards or customer requirements, and 15 % (226/922) were unsatisfactory due to the contamination of Coliforms bacteria (74%), *Legionella* (8%), Total plate count (6%), *E.coli* (5%), Coagulase positive Staphylococci (2%), and other bacteria (3%).
- 39 % of ice cube samples (192/493) from restaurants and bars were found to be contaminated by faecal bacteria, such as coliforms and *E. coli*.

Quality Management System

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

In 2024, with financial support of UNIDO, LEFS broadened its accreditation scope by adding five more parameters to its five existing food microbiology parameters, along with four additional parameters in water microbiology, as detailed below.

Parameters – Food / Environment	Method	New (Yes/No)
Culturable microorganisms at 30°C	NF EN ISO 4833-1	No
Presumptive *Bacillus cereus* at 30°C	NF EN ISO 7932	Yes
Coliforms bacteria	NF ISO 4832	No
Enterobacteriaceae	NF EN ISO 21528-2	No
Escherichia coli	NF ISO 16649-2	No
Yeasts and Moulds	NF V 08-059	Yes
Listeria monocytogenes	NF EN ISO 11290-1	Yes
Listeria monocytogenes	NF EN ISO 11290-2	Yes
Salmonella	NF EN ISO 6579-1	No
Coagulase positive Staphylococci	NF EN ISO 6888-1	Yes
Parameters – Water	**Method**	
Culturable microorganisms at 22°C	NF EN ISO 6222	Yes
Culturable microorganisms at 36°C	NF EN ISO 6222	Yes
Coliforms bacteria	NF EN ISO 9308-1	Yes
Escherichia coli	NF EN ISO 9308-1	Yes

Table 10: LEFS scope of accreditation with ISO17025:2017

4.2.3 Research Programs - Major Achievements in 2024

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

Diarrheal diseases are a leading contributor to the global disease burden in children, with Cambodia having one of the highest child mortality rates in Southeast Asia, where 6% of childhood deaths are attributed to these diseases. While unsafe water and poor sanitation have long been recognized as the primary causes, recent studies indicate that foodborne diseases also contribute significantly to the burden. In Cambodia, the specific pathogens responsible for most diarrheal cases remain largely unidentified, and comprehensive data on foodborne pathogens is scarce. Acute diarrhea is widespread across all socio-economic groups, suggesting that factors beyond poverty, such as unsafe food, also play a critical role.

Cambodia's food safety efforts have primarily focused on chemical contamination, but there is a need to raise awareness about the role of microbial pathogens in diarrheal diseases. Most food in Cambodia is purchased from informal markets with limited regulation and poor sanitation, making it essential to focus on these settings to reduce foodborne diseases.

The primary goal of this project is to reduce the bacterial contamination of vegetables, thereby lowering the risk of foodborne diseases. Specific objectives include identifying critical control points, developing targeted interventions, and implementing engagement programs to promote better food safety practices.

The LEFS was responsible for collecting clinical data on children under 15 with diarrheal diseases from hospitals in Phnom Penh, Battambang, and Siem Reap. Through sample analysis, *E. coli* and *Salmonella* were identified as the primary bacterial pathogens associated with vegetable-borne diseases. Sampling sites included farms, distribution centers, and market vendors in Siem Reap, focusing on high-risk vegetables such as cucumbers, lettuce, and tomatoes, as well as environmental samples from food contact surfaces. The samples were analyzed using protocols such as manual culture, PCR and gel electrophoresis for *E. coli*, and Real-Time PCR for *Salmonella*. The isolated *E.coli* and *Salmonella* strains were sent to US partner for Whole Genome Sequencing for in-depth analysis.

Results

A total of 704 samples were gathered between April and November 2022, including 480 vegetable samples and 224 environmental samples. These were collected from various points along the vegetable supply chain, such as farms, distribution center (DC) vendors, and market vendors.

The occurrence of Salmonella was significantly higher during the rainy season compared to the dry season ($P<0.05$). However, no substantial differences in Salmonella prevalence were detected across different stages of the supply chain.

E. coli was found at a notably high rate of 43%, exceeding the 5% prevalence of *Salmonella*. Its presence was significantly greater in lettuce and food contact (FC) surfaces compared to other sample types ($P<0.05$). However, seasonal variations did not have a significant effect on *E. coli* detection rates.

The in-depth analysis by whole genome sequencing found a total of 23 different serotype identified among 36 isolated in Siem Reap province. These include Thompson (n = 6), Weltevreden (n = 5), Paraptyphi B var. Java (n = 3), Albany (n = 2), Javiana (n = 2), as well as Hvittingfoss, Stanley, Virchow, Cerro, Corvallis, Derby, Farmsen, Infants, Johannesburg, Kentucky, Litchfield, London, Mgulani, Poona, Typhimorium, Wandsworth (n = 1, each) (the detail results can be found in the article listed below).

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)

AMR Working Group - IPC

The Head of LEFS is a member of the AMR Working Group at IPC, which was established in early 2023. In this role, I regularly attend meetings, contribute AMR-related insights, and engage in discussions to explore collaborative opportunities among various IPC units.

AMR Symposium

The Head of LEFS served as a co-organizer of **the 3rd International RAPID Symposium**, held at IPC, Phnom Penh, Cambodia, from December 10 to December 11, 2024. The symposium focused on the theme **"One Health Perspective to Address AMR in the Asia-Pacific Region."**

RAPID (R&D Alliance for Preparedness of Infectious Diseases) is a newly established research and development alliance initiated by scientists in the Pasteur Network Asia Hub. It aims to promote international R&D collaborations in infectious disease research, with a particular emphasis on Antimicrobial Resistance (AMR).

A total of 12 speakers were invited to the symposium, including four representatives from the Institut Pasteur (IP) Network, such as IP Korea, IP Hong Kong, and IP Nha Trang. The remaining eight speakers came from the Ministry of Health, the Ministry of Agriculture, Forestry and Fisheries, universities, hospitals, and IPC. Around 30 participants attended the event.

4.2.4 Support to National Authorities

For several years, the LEFS has supported different national authorities in Cambodia, including the Food and Drug Department of the Ministry of Health, and the National Animal Health and Production Research Institute, attached to the Ministry of Agriculture, Forestry and Fisheries.

In 2023, as part of a national monitoring program, the Ministry of Health sent 470 samples to LEFS. These samples were obtained through product hygiene campaigns of industrial foods imported and exported. Also as part of a national monitoring program, the Ministry of Agriculture, Forestry and Fisheries sent 122 sample to LEFS.

The head of LEFS is a member of the Foodborne Outbreak Response Team (FORT), coordinated by the CDC-MOH in Cambodia. In addition to supporting testing services during outbreaks, we contribute to drafting food safety policies, reviewing standard operating procedures (SOPs), and participating in risk assessment evaluation meetings related to outbreak events.

4.2.5 Teaching and Training

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

University	Number of students	Program Year	Period (Month)	Date
	2	Year 3	1.5	17/06/2024–31/07/2024

Royal University of Phnom Penh	1	Year 3	1	01/10/2024–31/10/2024
International University	3	Year 3	1	01/10/2024–31/10/2024 01/11/2024–30/11/2024
Institute of Technology of Cambodia	5	Year 3	1	01/08/2024–31/08/2024 02/09/2024–30/09/2024

Table 11. Internship Students at the LEFS in 2024

4.2.6 Outlook for the Upcoming 3-5 Years

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

- Maintaining the lab accreditation status;
- Increasing laboratory visibility/recognition with the public;
- Extend heavy metal testing from water matrix to other matrix such as food and soil using GF-AAS and also extend to other metal
- Set up pesticide and antibiotic residue analysis by using LC-MS/MS and GC-MS/MS
- Extension of accredited scopes to heavy metal and pesticide residue testing
- Develop increasing cooperation with internal and external partners for research projects.

4.2.7 Scientific Publications 2024

Note: *The name of authors from the Institut Pasteur du Cambodge are underlined*

1. **Genomic Diversity and Potential Transmission and Persistence of Salmonella in the Cambodian Vegetable Supply Chain**.
   Salazar A, Sreng N, Peng C, Fu Y, Nawrocki EM, Chung T, Vipham J, Dudley EG, Kovac J. J Food Prot. 2025 Feb 3;88(2):100447. doi: 10.1016/j.jfp.2024.100447. Epub 2025 Jan 4.

4.3 Vaccination Service

4.3.1 Functional Structure

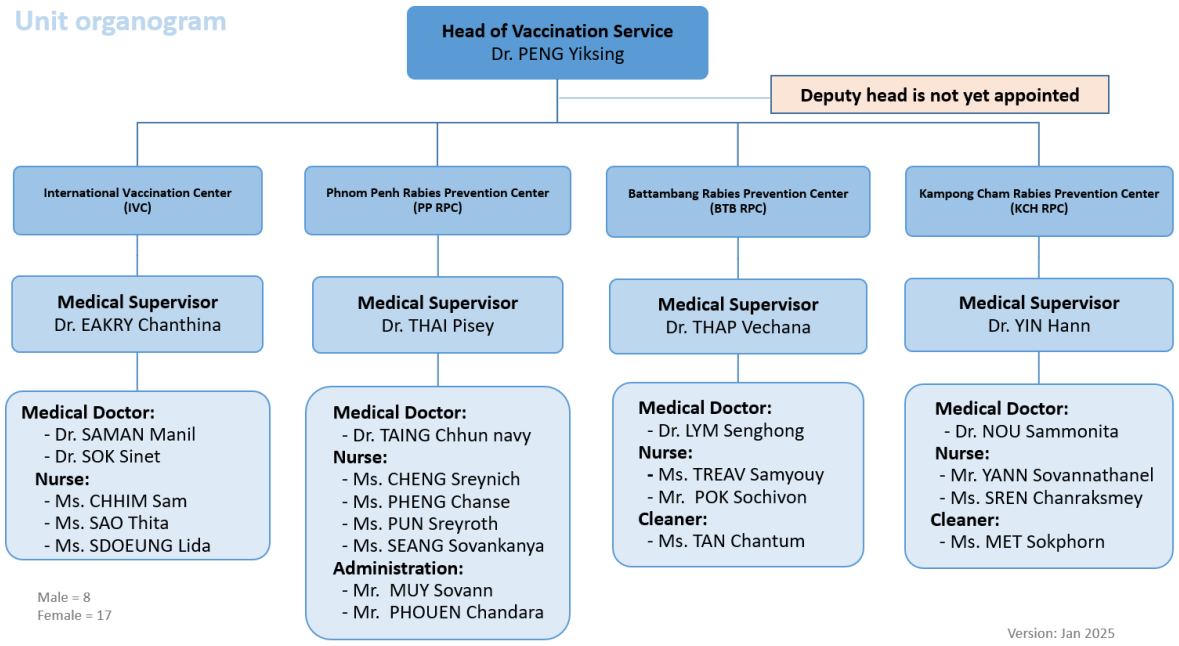


Figure 28. Vaccination Service Organogram

The unit has 25 team members based in Phnom Penh and two provinces: ten medical doctors (including the head of the Vaccination Service and four medical supervisors), eleven nurses, two administration staff, and two hygiene personnel.

4.3.2 Rabies Prevention Centers

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

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

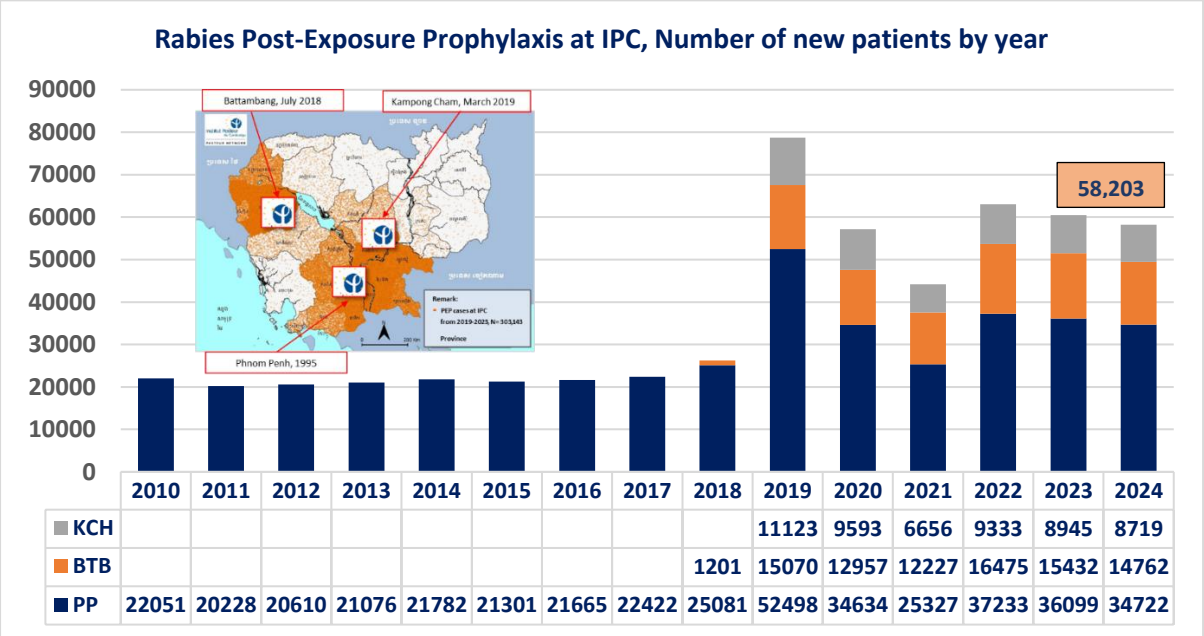
Diagnostic tests on brain samples of biting animals are done by the Virology Unit, and timely results are provided free of charge to the patients even if samples are shipped from our two provincial PEP centers.

Rabies Prevention Centers Activities in 2024:

- Provided rabies post-exposure prophylaxis to 58,203 patients.
  - 34,722 patients received rabies PEP at the Rabies Prevention Center in Phnom Penh
  - 14,762 patients received rabies PEP at the Rabies Prevention Center in Battambang
  - 8,719 patients received rabies PEP at the Rabies Prevention Center in Kampong Cham
- Despite efforts from MoH-CDC and GDAPH to eliminate human rabies through dog mass vaccination campaigns in various areas in 2023 & 2024 (PP, Kandal, and Battambang), the number of patients who received PEP at IPC remained stable compared to 2023.
- A total of 187 animal heads were tested by immunofluorescence for rabies virus at the Virology Unit. 121 samples (68.7%) were positive for rabies:

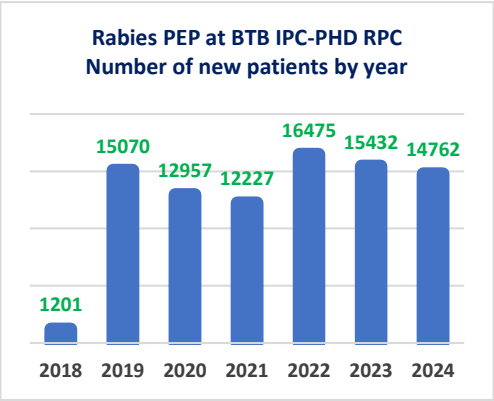
- 70.8% of all dog samples (119/168) were positive for rabies,
- 11.7% of all cat samples (2/17) were positive for rabies.

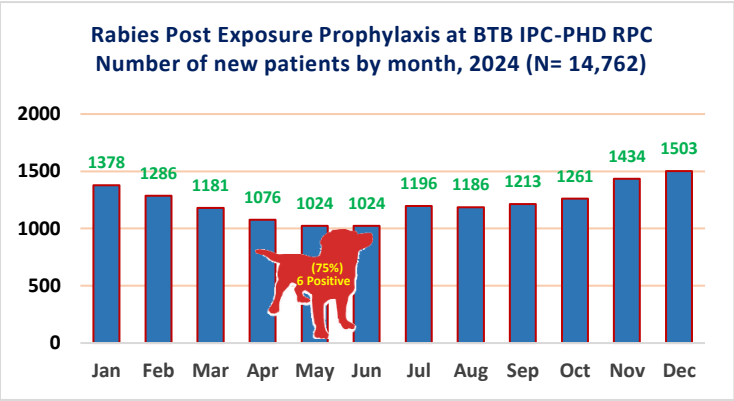
- The information on animal rabies diagnosis is communicated to MoH-CDC, WHO, and GDAPH regularly.



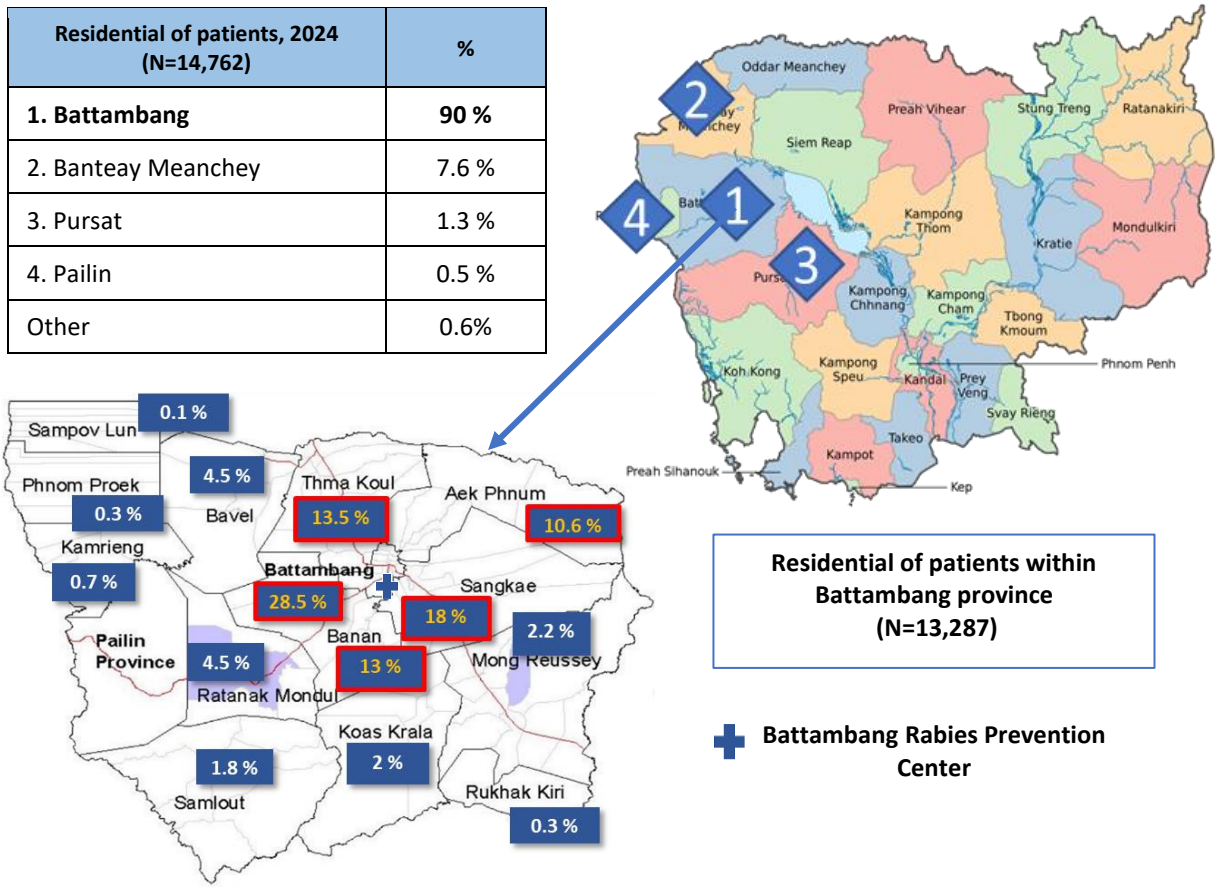
4.3.2.1. Battambang Rabies Prevention Center

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 extend rabies PEP coverage to Battambang and 5 other neighboring provinces.





Residential of patients, 2024 (N=14,762)	%
1. Battambang	**90 %**
2. Banteay Meanchey	7.6 %
3. Pursat	1.3 %
4. Pailin	0.5 %
Other	0.6%



4.3.2.2. 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 was initially located within Kampong Cham Provincial Hospital and uses a temporary building provided by the hospital. This center extends rabies PEP coverage to six other provinces of Northeast Cambodia. The agreement between IPC and KC-PHD for the construction of a permanent Rabies Prevention Center in Kampong Cham was signed on 26th April 2023.

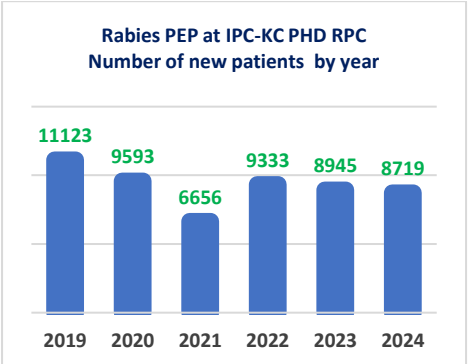
On Monday, 6th May 2024, the *Institut Pasteur du Cambodge* (IPC) inaugurated with the Ministry of Health our new Rabies Prevention Center in Kampong Cham. This new IPC-KC PHD Rabies Prevention Center replaces the previous one installed in 2019. The ceremony was held under the presence of HE. Dr. Ngov Kang, Secretary of State, MoH, HE. HAN Kosal, the Deputy Provincial Governor of the Kampong Cham Province, the Directors and Deputy Directors of the Provincial Health Department (PHD), and the IPC directions.

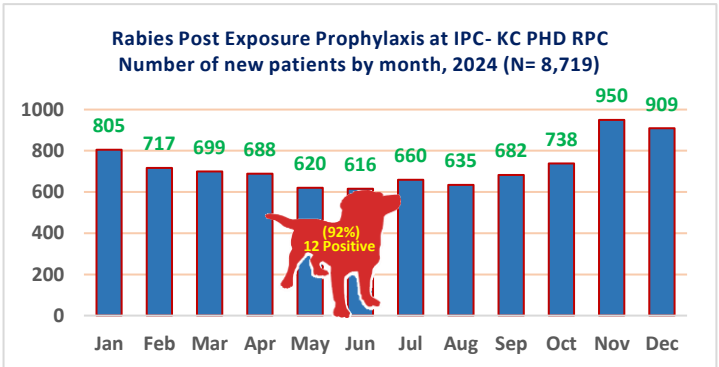
The new center is a single-storey building of 180 m^2 (12 meters wide by 15 meters long), it's designed based on IPC teams' experience acquired over several years in this expertise. The center is more visible and convenient for IPC to provide PEP and vaccination services in the best reception and safety conditions.

HE. Dr. NGOV Kang, Secretary of State, MoH

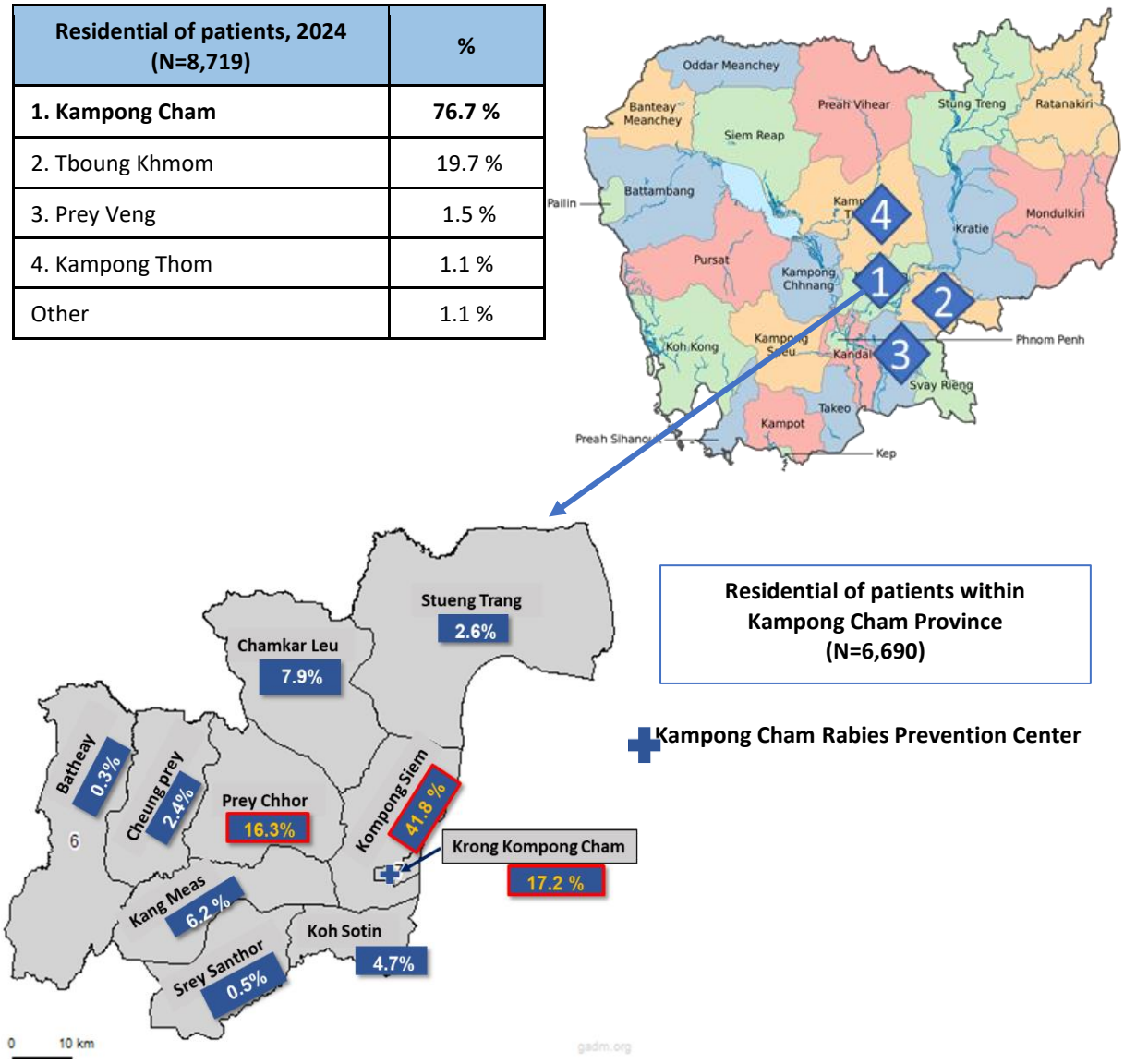
Pro. André SPIEGEL, Director of IPC

Inauguration of the new IPC-KC PHD Rabies Prevention Center in May 2024





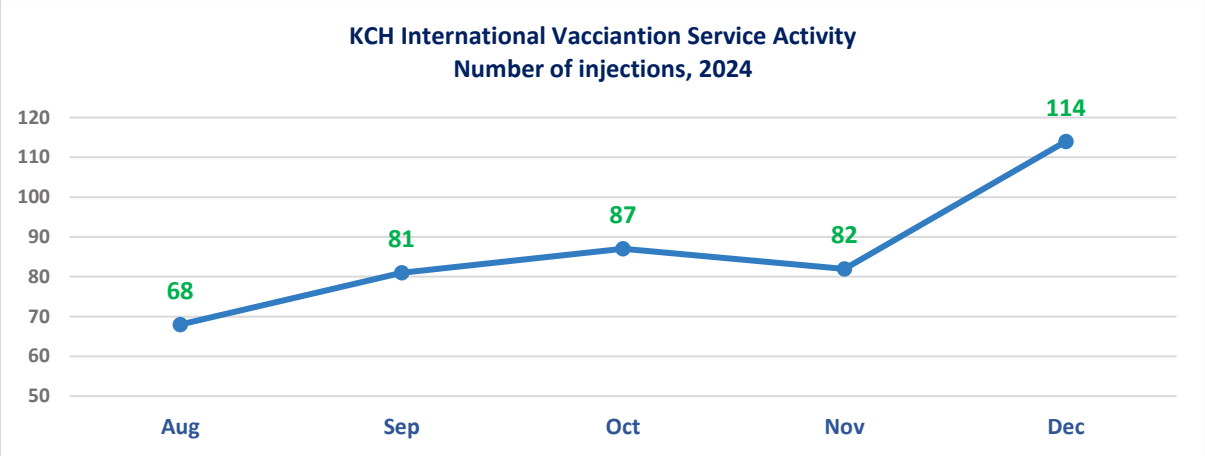
Residential of patients, 2024 (N=8,719)	%
1. Kampong Cham	**76.7 %**
2. Tboung Khmom	19.7 %
3. Prey Veng	1.5 %
4. Kampong Thom	1.1 %
Other	1.1 %

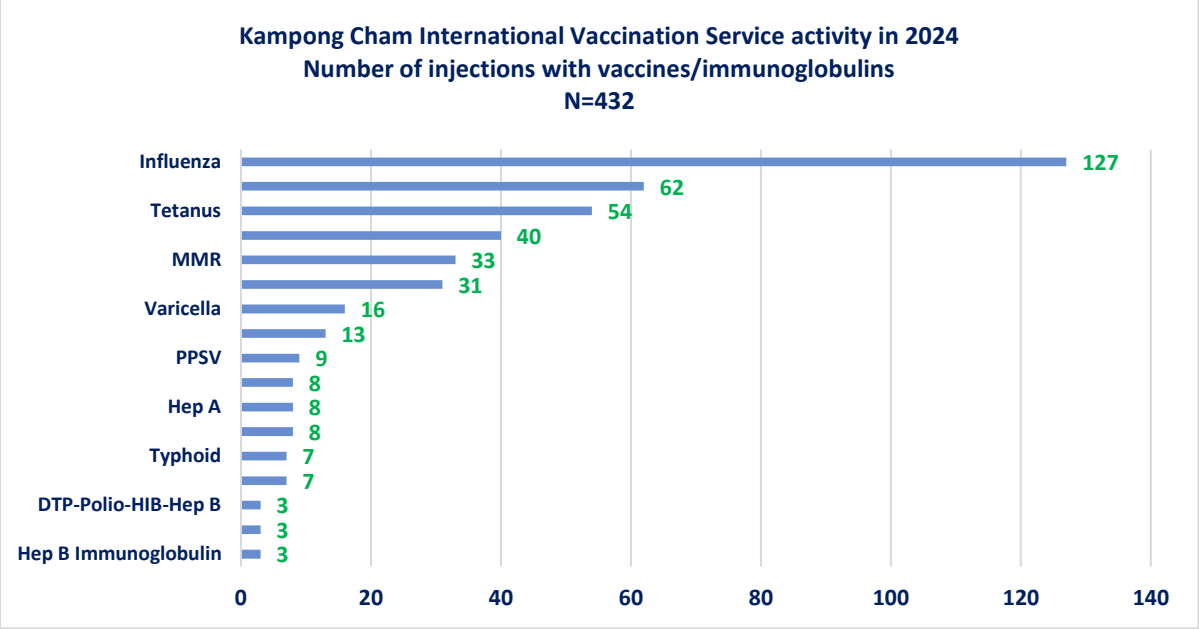


4.3.2.3. Kampong Cham International Vaccination Service

Answering the proposal from the Ministry of Health and Kampong Cham Health Department to *Institut Pasteur du Cambodge* concerning the possibility of setting up an International Vaccination Service in Kampong Cham for the public health benefit of the local people. On 1st August 2024, the International Vaccination Service was integrated with the existing IPC-KC PHD Rabies Prevention Center in Kampong Cham. The setup and operation of this service activity follow all the standard operating procedures that have been operated at the International Vaccination Center in Phnom Penh.

This new service activity opens the opportunity to bring IPC's quality vaccination service against other vaccine-preventable diseases to Kampong Cham province for the benefit of the public. Currently, a wide range of vaccines including those that are part of the national immunization program and other essential optional vaccines are available at Kampong Cham Rabies Prevention Center.





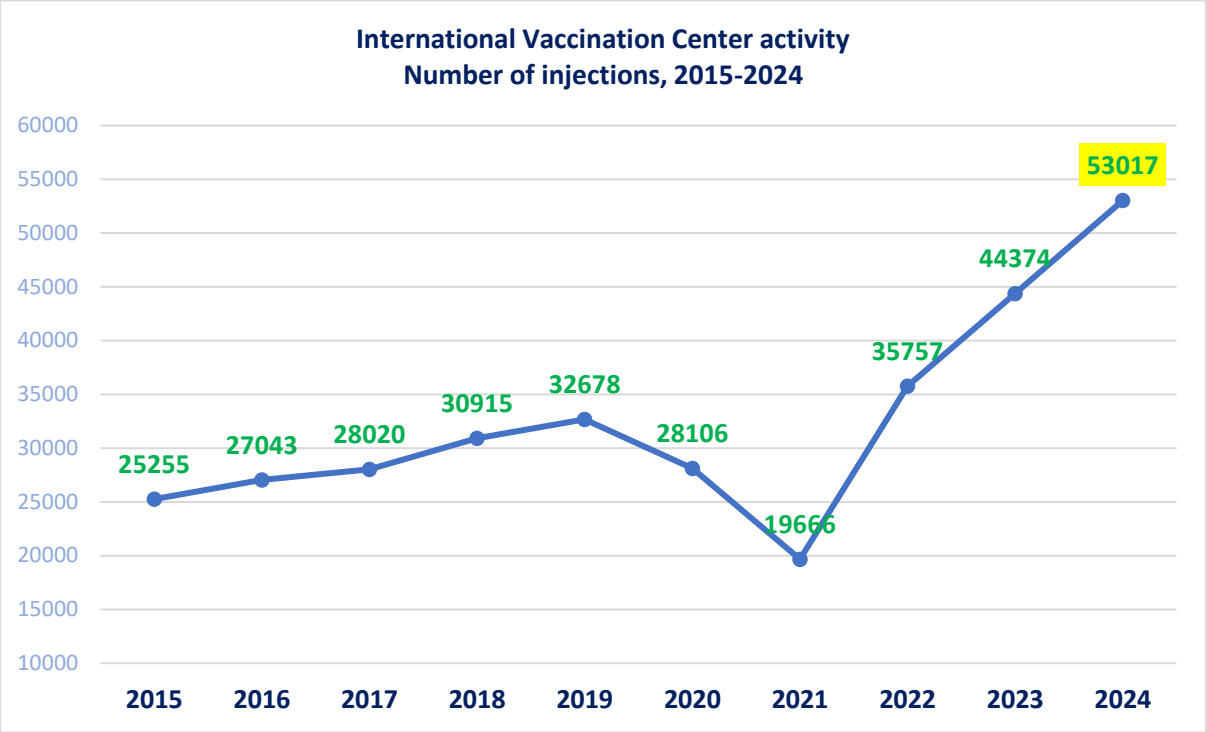
4.3.3 International Vaccination Center

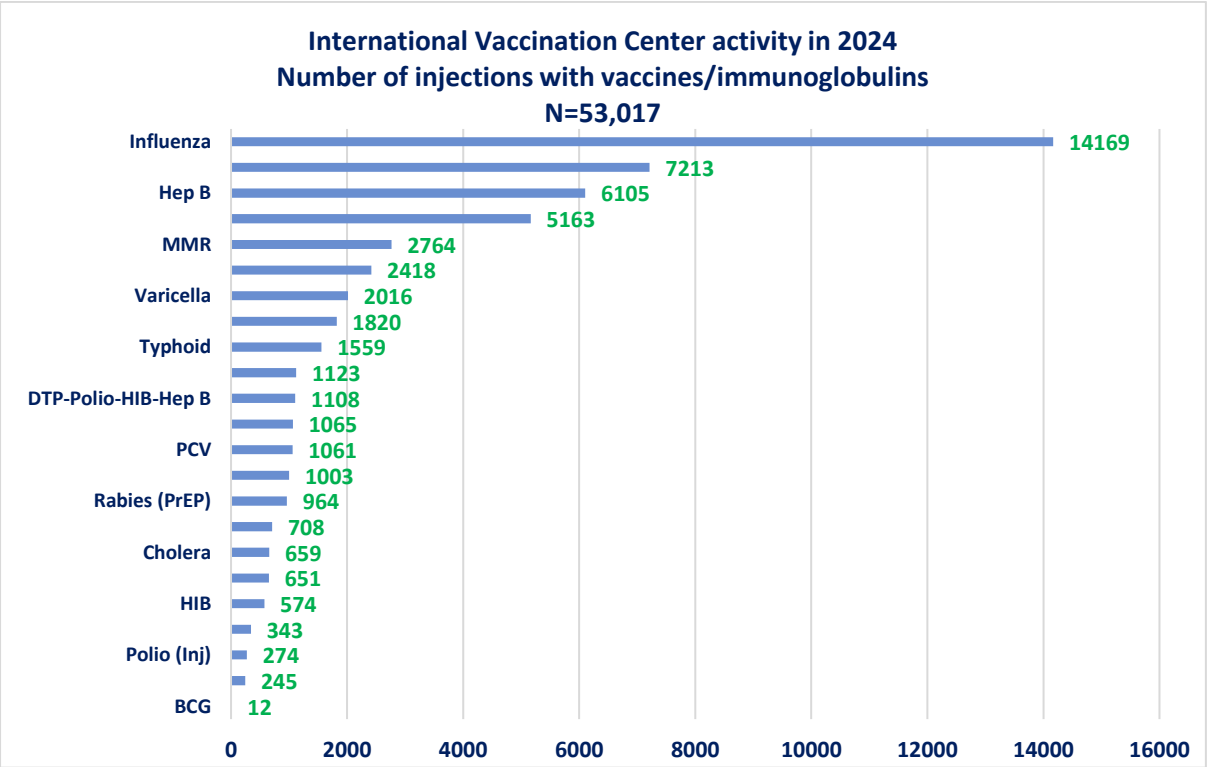
The International Vaccination Center at the *Institut Pasteur du Cambodge* has a medical team of 6 full-time staff under the responsibility of the head of the Vaccination Service. One additional doctor was recruited in 2024 to support the team in response to the significant increase in the number of clients. In 2024, our cold chain management quality was also strengthened, in addition to the existing web-based live remote monitoring and alert notification system, 7 WHO-prequalified professional fridges for vaccine storage were purchased and installed at our 3 vaccination center sites to ensure that the quality of our vaccines is always maintained according to the standard operating procedures for vaccine storage.

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.

The International Vaccination Center once again achieved record-breaking and remarkable results in 2024 the global activity of the International Vaccination Center in 2024 increased by 19.4% compared to 2023 (53017 vs 44374). Several key factors contribute to these impressive results including the

proper procurement and business plan, good accreditation of IPC, quality of the service, and effective communications of the International Vaccination Center.





4.3.4 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 provides rabies PEP to the public at an affordable price, contributes to the development of the National Rabies Elimination Strategy and the development of the National

Rabies Surveillance Guideline, technical assistance to prepare Cambodia's application to Gavi for Rabies Vaccine Program Support, participates in EIC (education, information, and communication) activities, and is a national reference training center for rabies PEP.
The Vaccination Service cooperates with several national authorities and international agencies (MoH, US-CDC, *Institut Pasteur* in Paris, WHO, GDAPH, FAO, CIRAD, GIZ, and others) to contribute to rabies elimination programs and research studies, especially on rabies.
IPC's International Vaccination Center also plays a crucial role in supporting the Cambodia Ministry of Health with technical advice on immunization practices and applied policy regarding vaccination programs.

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

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.

Contributing to the fight against rabies in Cambodia

As an ASEAN member state, Cambodia has committed to eliminating rabies by 2030. To achieve this milestone, three main action plans to fight against rabies have been developed by IPC in collaboration with the Ministry of Health:

1- Increasing accessibility to rabies PEP by improving the visibility of our centers.
2- Raising awareness about rabies through education, information, and communication activities.
3- Supporting the MoH in the rabies elimination mission by participating in developing the National Rabies Elimination Strategy.

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.

4.3.6 Scientific Publications 2024

Note: *The name of authors from the Institut Pasteur du Cambodge are underlined*

1. **Evaluation of one year immunity following rabies post-exposure prophylaxis in dog bite cases.**
   Ya N, Auerswald H, Touch S, In S, Yun C, Thai P, Sann S, Heng B, Leng C, Duong V, Peng YS, Ly S, Cantaert T. NPJ Vaccines. 2024 Nov 27;9(1):237. doi: 10.1038/s41541-024-01030-8.

5 Technical Platforms

5.1 BSL3 Laboratory

2024 at IPC's BSL3 Laboratory: A Year of keeping Innovation and Collaboration

As Cambodia's sole BSL3 facility, IPC has been pivotal in handling pathogens like avian influenza, Mycobacterium tuberculosis, SARS-CoV-2 and M pox, contributing significantly to both diagnostic and research capabilities. This role has significantly bolstered both diagnostic and research capabilities within the region. In 2024, the Institute Pasteur Cambodia's (IPC) BSL3 laboratory remained a beacon of excellence in infectious disease research. As IPC's frontline in handling high-risk pathogens, the BSL3 lab has been instrumental in advancing public health safety and scientific understanding of infectious diseases.

The BSL3 laboratory's governance is defined by a clear organizational structure emphasizing specialized roles and collaborative decision-making (Figure 29). The facility management is a cooperative effort spearheaded by the General Scientific Manager and the Technical Manager. The Scientific Manager is tasked with the rigorous oversight of biosafety protocols, ensuring that the laboratory's handling of pathogens and execution of procedures are in strict adherence to safety standards. This is carried out in close collaboration with the scientific supervisors of each module, enabling a tailored approach to biosafety that aligns with the specific requirements and risks associated with the various research activities and pathogens handled in the lab. Key to the laboratory's operation is the Technical Manager, who ensures the technical proficiency and operational integrity of the laboratory's functions. This role involves developing and implementing stringent biosecurity protocols to prevent unauthorized access to pathogens, along with overseeing the regular maintenance, calibration, and validation of laboratory equipment and infrastructure to guarantee that they meet the necessary standards and regulations for safe and effective operation. These pivotal management roles are supported by specialized teams that manage waste, oversee quality, and include an occupational health doctor. Each BSL3 module is managed by two dedicated supervisors, responsible for overseeing pathogen-specific protocols, and maintaining biosafety standards, ensuring that all laboratory activities are executed with the highest safety and efficiency. Decision-making within the BSL3 team is likely collaborative, informed by expertise from each section, ensuring that all decisions are well-informed, comprehensive, and conducive to maintaining the highest quality of laboratory operations.

The General Scientific Manager of BSL3, Mrs. Heidi AUERSWALD, left her position since October 2024.

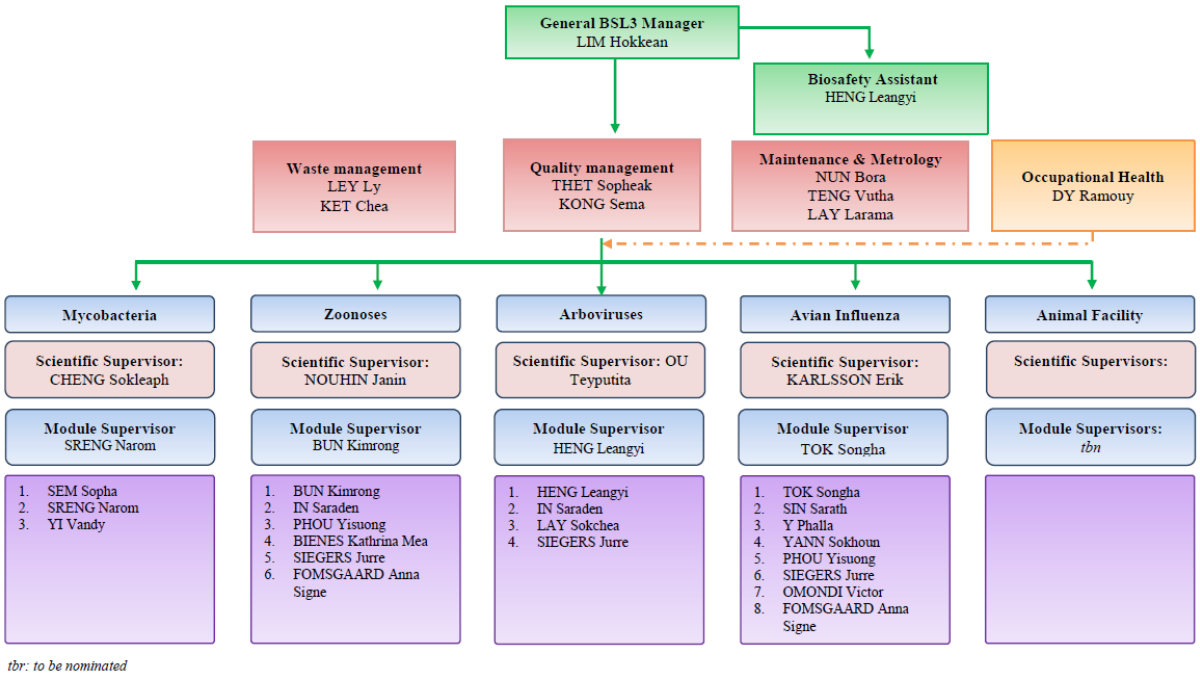


Figure 29: Organigram of the BSL3 Management

Following the renovation at the end of 2023 of the ducting ventilation and cooling system, specifically the chiller unit, significant improvements have been observed in terms of operational comfort and energy consumption. The upgraded system has enhanced the overall working environment by providing better control over temperature and airflow, leading to increased comfort levels for occupants within the facility, throughout 2024.

Moreover, the efficiency enhancements resulting from the renovation have translated into notable energy savings. The optimized operation of the chiller unit has enabled a more effective cooling process while consuming less energy, ultimately contributing to improved sustainability and cost-effectiveness in the building's operations.

Following the installation of waste treatment system funded by European Union (EU) in 2023, the Steam sterilizer *(Matachana S1000)* was installed in May 2024 for IPC's Biosafety Level 3 facility to enhance significantly our capabilities in life science and Health research on infectious diseases and emerging pathogens, to improve our overall operability and to mitigate risks to committee and Environment.

On October 30, IPC welcomed H.E. Igor Driesmans, Ambassador of the European Union (EU). The state-of-the-art tools are essential to the safe handling of biohazardous waste from our activities.

In 2024, IPC concentrated its efforts on enhancing staff training, focusing specifically on both new and existing employees to ensure they were thoroughly educated in biosafety, biosecurity practices, and the implementation of ISO 35001:2019 standards.

Internal auditors were dedicated to ensuring the organization's compliance with the stringent requirements of ISO 35001:2019, particularly in the context of Biosafety Level 3 (BSL3) laboratories. This certification requires rigorous standards for laboratory environments where high-risk biological materials are handled.

5.1.1 Support for National Authorities

In 2024, IPC's BSL3 laboratory continued to play a crucial role in supporting national authorities, particularly in fulfilling international standards on biosafety and biosecurity. IPC's commitment to these standards is evident in its ongoing support for government actions related to the construction of other BSL3 facilities and general laboratory enhancements. This support is exemplified through joint training initiatives, such as those conducted under the EU Project 81, which focused on enhancing regional biosecurity capabilities.

The EU Project 81, also known as the BIOSEC project, aims to enhance biosecurity in the ASEAN region by strengthening national capabilities to prevent, detect, and respond to biological threats. Key objectives include improving border control for biological threats, enhancing regulatory controls over high-risk biological materials, and building capacity for best practices in biological materials storage and transport. The project focuses on collaborative efforts, including training workshops and the development of a network laboratory testing capability tool. Institute Pasteur Cambodia (IPC) actively supports the objectives of the BIOSEC project. IPC's involvement includes participation in multiple workshops organized by the Ministry of Defense's NACW department, contributing to the project's goals of improving laboratory capacity and biosecurity measures. These efforts align with IPC's commitment to enhancing regional biosafety and biosecurity standards.

Furthermore, IPC has extended its support to the Ministry of Defense, particularly in implementing a National Inventory for High-Risk Biological Agents. This collaboration underscores IPC's role as a leader in biosafety and biosecurity, contributing significantly to national and regional efforts in managing biological risks.

IPC is also involved in the construction of BSL3 facility to provide technical assistance at National Institute of Public Health NIPH). This is an achievement, as it enhances the biosafety and biosecurity in Cambodia by mentoring the process of design and construction of this new facility.

For the constructions for projects in the Calmette Hospital, IPC also contributed its expertise to the construction of the Cyclotron Building which houses equipment for producing radioactive isotopes for medical treatments. This facility requires strict safety and regulatory oversight, and IPC's technical assistance focused on ensuring compliance with safety standards, particularly for radiation safety. On the other hand, IPC also supported the renovation of the Bone Marrow Transplant (BMT) facility. The renovation aimed to improve cancer treatment infrastructure, with IPC providing guidance on the biosecurity and safety standards essential for patient care in this specialized setting.

5.1.2 Teaching, Training and Collaboration

Educational Initiatives and Skill Development at IPC's BSL3

This year, during the maintenance period, IPC conducted comprehensive drills and training sessions on emergency response procedures, focusing on two key areas: spills and fire emergencies. These drills were designed to ensure the preparedness and safety of IPC's employees preparedness and for external collaborators, such as NIPH (National Institute of Public Health) and the Institute Pasteur of Laos.

In addition to its technical assistance in construction projects, IPC contributed to the development of human resources by providing teaching courses on Biosafety and Biosecurity at the University of Health Sciences. By educating students on these critical topics, IPC helped to build a future workforce skilled in maintaining high safety standards in healthcare facilities, research, and laboratory settings for Biomedical Engineering Associate Degree Program in the university (IPC is assigned as the

referential expertise by Ministry of Health). This initiative not only strengthened the students' knowledge base of the students but also contributed to the long-term development in the country.

5.1.3 Outlook for 2025

Enhancing Operations and Ensuring Compliance

A significant focus for 2025 will be on achieving ISO 35001:2019 certification. The implementation of the Quality Management System according to ISO 35001 standards will be carrying out across all processes in the laboratory. The laboratory has completed the necessary documentation preparation in 2023 and is now moving towards the application and certification process. This step is crucial for demonstrating IPC's commitment to global standards and requirement of ASEAN in biorisk management.

Aligned with the 18-month maintenance schedule, the BSL3 lab was in May 2024 and is set for its regular maintenance in November 2025. This routine maintenance is critical for ensuring the BSL3's operational integrity and safety compliance, and this applies equally to its equipment

The plans for 2025 reflect IPC's commitment to maintaining the highest standards of biosafety and operational excellence, while also preparing for future challenges and advancements in the field.

5.1.4 Vision for the Future

IPC's BSL3 Laboratory's Blueprint for Advancement and Innovation

Looking ahead, IPC's BSL3 lab is aligned with ambitious objectives, as outlined in its strategy. Over next three years, the focus will be on enhancing biosafety protocols, particularly by updating guidelines to meet top international standards and acquiring the ISO35001:2019 certification. Concurrently, IPC will invest in advanced diagnostic technologies and initiate plans for a new BSL3 construction. However, it has been challenging to find a suitable candidate to fulfill the position of General Scientific Manager as Virologist for BSL3 in Cambodia. The current General BSL3 Manager is studying his PhD on "Enhancing Laboratory Safety in LMICs such as Cambodia: Implementing and Upgrading BSL3 Biosafety Standards".

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

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

5.2 Biobank

5.2.1 Background

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

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

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

The current users of the biobank are the Virology Unit and the Epidemiology and Public Health Unit. All samples are stored in -80°C freezers. In addition, we now have 47 units of such freezers and in the future, some samples will be stored in liquid Nitrogen (N_2). IPC also has its own liquid nitrogen generators for daily production of around 100 liters per day which meets the demand of use for all the laboratories at IPC.

All of the freezers are installed in a purpose-designed room equipped with air conditioners working permanently. This room is only accessed by a group of authorized personnel, which means that the entry of the biobank rooms is strictly controlled. The freezers are constantly monitored through the use of monitoring sensors (the Oceasoft system).

5.2.2 Functional Structure

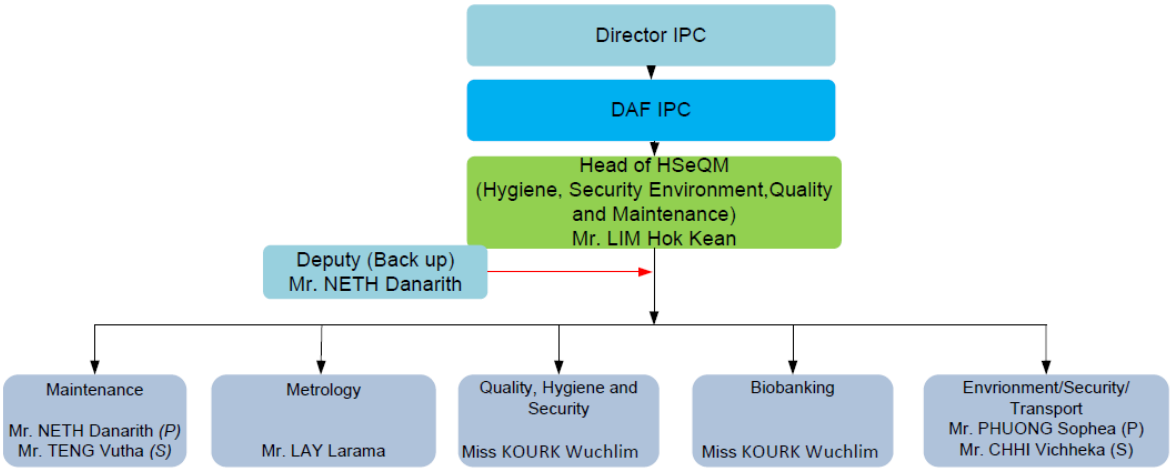


Figure 30: Organigram of the Biobank Management

5.2.3 Biobank Database

At present, there are 16 research projects registered in the biobank software with the total number of 56150 samples as shown in more detail in the Table below:

Collection/Project	No. of samples	Nature of samples	Storage
LEFS			
Food Safety Innovation Lab	674	*E. coli* *Salmonella*	-80°C freezer (HSM-04) Freezer code: I/01599
LBM			
Aspergilloma in Cambodia	3051	Serum Aspergillus	-80°C freezer (HSM-04) Freezer code: 439
Entomology			
JEV vectors Behaviours (Comacross)	0	N/A	N/A
Immunology			
Study of Dengue-like illness in Kampong Thom	**0**	N/A	N/A
Virology			
ECOMORE 2	6421	Serum Saliva	-80°C freezer (HSM-04) Freezer code:
Biodiversity convervation to mitigate the risks of emerging infectious diseases	0	N/A	N/A
Immuno PEP follow up 2019	234	Serum	-80°C freezer (VIR-02) Freezer code: 1324
RAB00056 IM/ID (Sanofi study)	26	Serum	-80°C freezer (VIR-02) Freezer code: 1324
Rabies surveillance	14050	Ammon's horn Spinal bulb	-80°C freezer (VIR-02) Freezer code: 1324
Rodents as Reservoir for Hepatitis E Virus (HEV), Arenavirus and Other Rodent-borne Viruses and risk assessment of infection in human in Cambodia	1938	Kidneys, Swab, Urine, Heart, Lung, Liver, Ectoparasite, Blood clot, blood serum, Pool organ	N/A
Epidemiology and Public Health			
Toward an integrated surveillance of potential zoonotic Betacoronaviruses in the wild animal value chains of Cambodia	0	N/A	N/A

HEPEDIAC- Pilot therapeutic study of DAA treatment for children and adolescents with active HCV infection in Cambodia	517	Whole blood and plasma	-80°C freezer (HSM 04) Freezer code: 01609
Lowering Interleukin-1 Receptor Antagonist Concentrations after TB Treatment Onset: A proof of concept study in Cambodia and Ivory	303	Plasma Buffy coat	-80°C freezer (HSM-04) Freezer code: LILAC-TB
TB-Speed Output 2 - Severe pneumonia	1794	Plasma Stool Whole blood	-80°C freezer (HSM-04) Freezer code: 1551
Tenofovir As Prevention Of Hepatitis b Mother-to-child transmission	27509	Plasma Blood Red blood cell Buffy coat	-80°C freezer (HSM-04) Freezer code: 1475
Zika Sentinel Surveillance in prenatal care visit and maternity ward in Calmette Hospital (Phnom Penh, Cambodia)	16	Serum Urine	-80°C freezer (HSM-04) Freezer code: 439
Total samples	56533		

5.2.4 Action Plan

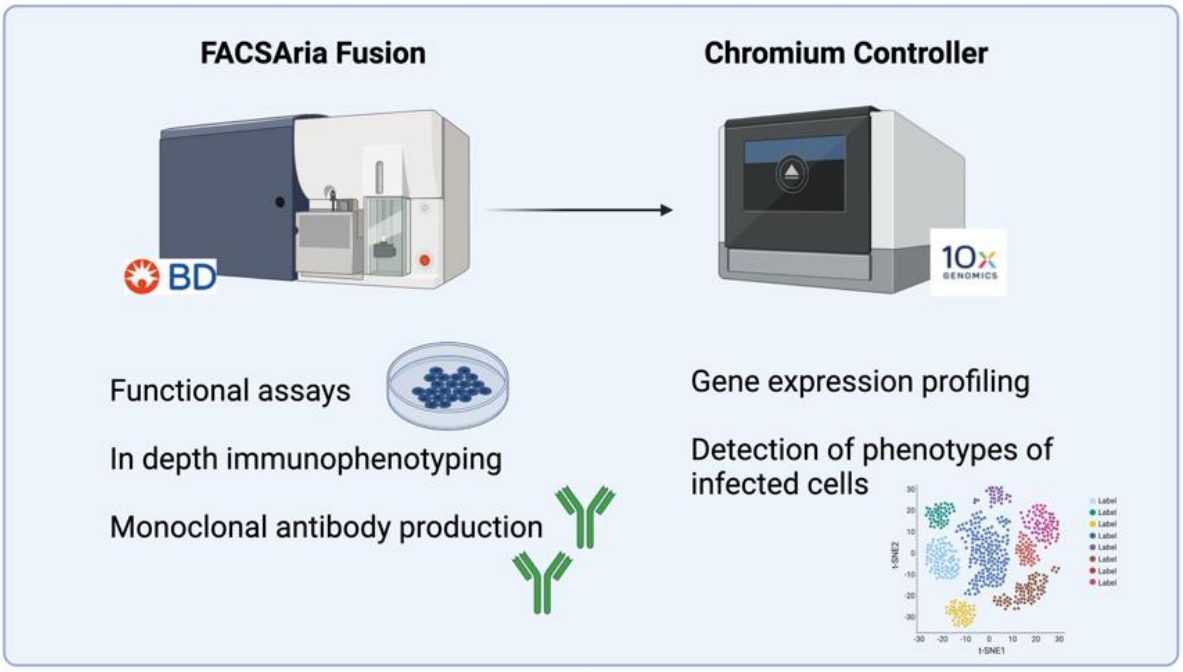
The biobank activities should be centralized under the responsibility of one person who is taking care of input and output of samples. The SOPs for the management of Biobank are currently being written and the Biobank policy will be developed and implemented in 2026.

5.3 Single Cell Analysis

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

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

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



5.3.1 Functional Structure

The platform is integrated within the Immunology Unit.

5.3.2 Research Programs – Major Achievements in 2024

In 2024, the platform was used for the following research projects:

1. Understanding the role of regulatory T cells in dengue virus infection
2. Evaluation of DENV-specific T cells during DENV virus infection

3. Evaluation of B cell receptor (BCR) and T cell receptor (TCR) genes in the context of dengue virus infection
4. Production of monoclonal antibodies directed to dengue and crimean congo hemorrhagic fever virus
5. Understanding of immune responses to chikungunya virus infection leading to chronic symptoms
6. Uncovering of the mechanisms governing IgG glycosylation
7. Assessment of skin immune responses to mosquito saliva and arbovirus infections
8. Identification of mechanisms leading to clinical protection from Plasmodium vivax infection
9. Understanding the mechanisms of plasmodium vivax receptor-ligand interactions involved in reticulocyte invasion

5.3.3 Research Programs – Outlook for 2025

In 2025, a continuation of the above mentioned research projects is envisioned. In addition, our pipelines for the production of monoclonal antibodies will be extended to other viruses of major concern in South-East Asia.

Perspectives

In the upcoming years, we aim to strengthen further the single cell analysis platform, with new projects on antibody discovery for relevant mosquito-borne infections, and expansion of the experimental pipelines to include Cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq).

The platform can be utilized to study interactions between host and pathogens of major health importance in Cambodia. The platform can help in the design, planning and execution of the experiments. Bioinformatics analysis are strengthened with hiring of a senior bioinformatician and the establishment of the BAIA Unit at IPC. Within Cambodia, we aim to setup novel collaborations with universities and other research entities. Indeed, we have an ongoing collaboration with the NIH-funded ICER (International Center for Excellence in Research) which is located at the National Center for Parasitology, Entomology and Malaria Control (CNM), Ministry of Health. Moreover, the platform aims to support research programs in Cambodia and in the region through workshops and training. A course in flow cytometry and cell sorting was held in September 2023 open to Cambodian students and other students from low-middle income countries.

5.4 Sequencing Platform

5.4.1 Functional Structure

Established in April 2021, the Sequencing Platform is a technical facility dedicated to strengthening - in transversal within IPC – research activities and public health responses related to genomic sequencing. Equipped with cutting-edge infrastructure, including a high-throughput next generation sequencer (Illumina MiSeq System) and a range of accessory instruments, the platform facilitates in-house advanced sequencing capabilities. From April 1, 2023, to June 30, 2024, it was co-led by Dr. Janin Nouhin and Dr. Nimol Khim. Effective July 1, 2024, Dr. Janin Nouhin serve as the sole Sequencing Platform Manager, dedicating 30% of time to this role, supported by a skilled research engineer, Mr. Vireak HEANG, and a laboratory technician, Mr. Sereyrith Saulim. Our activities encompass nucleic acid library preparation and next generation sequencing (NGS) using the state-of-the-art Illumina MiSeq System.

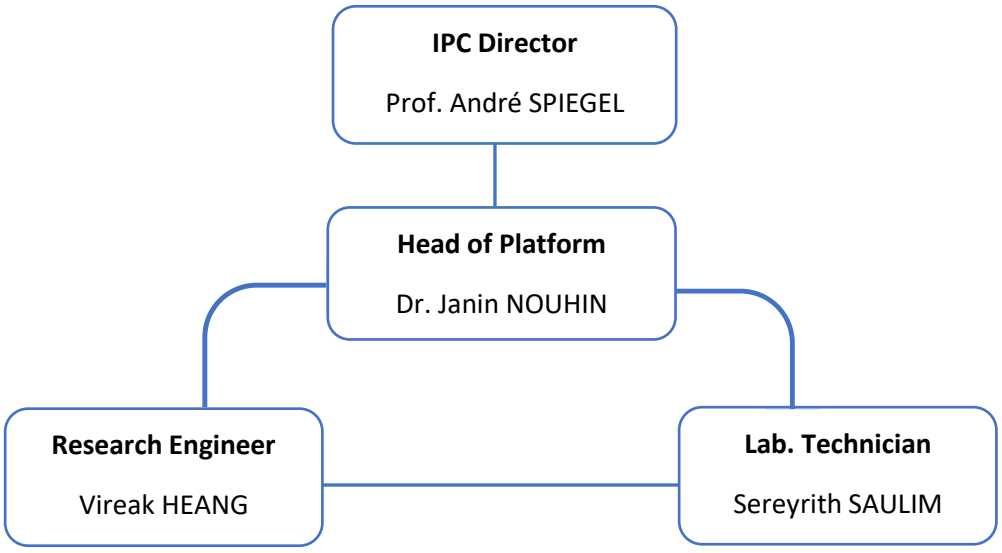


Figure 31. Sequencing Mini-Platform organogram, 2024

5.4.2 Research Programs – Major Achievements in 2024

In 2024, the platform team performed 26 runs of NGS service using Illumina Miseq (n=16) and Oxford Nanopore Technologies (ONT) (n=10). All sequencing run on Illumina MiSeq were fee-based users including Virology Unit (n=12), Malaria Research Unit (n=2), and DUKE-NUS Medical School (n=2), while the ONT runs were conducted in the framework of collaborative project funded by Bill and Melinda GATES foundation and led by Dr. Erik Karlsson. Distribution of NGS runs in 2024 is depicted in Figure 32.

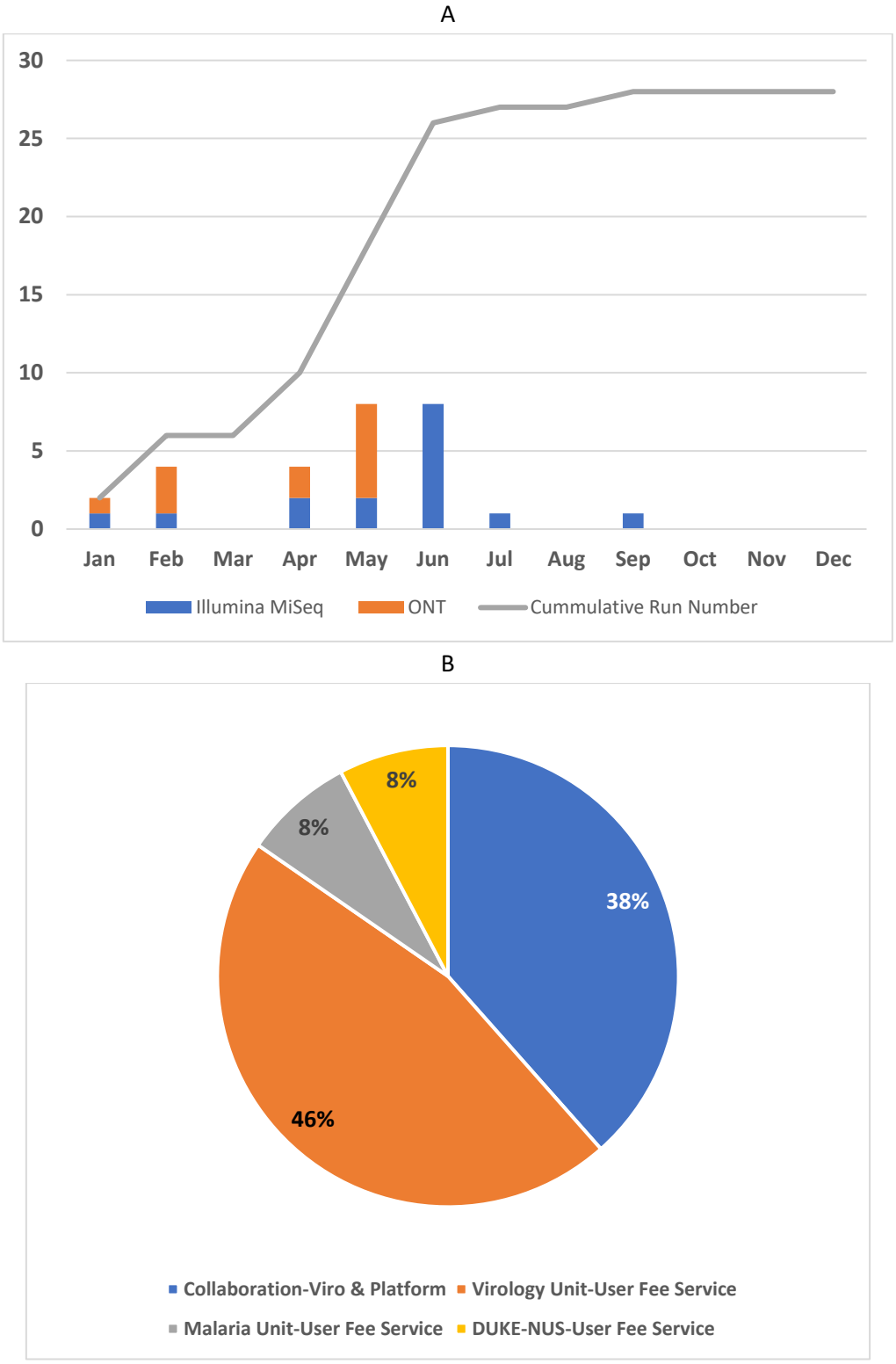


Figure 32. *Distribution of Next-Generation Sequencing runs in 2024. (A) Distribution of sequencing runs over monthly period in 2024. Monthly totals of Illumina MiSeq and ONT sequencing runs are indicated by blue and orange bars, respectively. Cumulative total is depicted by gray line. (B) Distribution of sequencing runs by category of users. Sequencing run performed in the framework of collaborative projects between the Sequencing Mini-Platform and the Virology Unit are in indicated in blue. Sequencing runs delivered to the Virology Unit as user fee services are indicated in orange. Sequencing runs provided to the Malaria Research Unit and DUKE-NUS are indicated in gray and yellow, respectively.*

5.4.2.1 Collaborative Projects

Project 1: Expanded Environmental Surveillance and Metagenomics for Zoonotic Risk/Prevention in Cambodian Live Animal Markets

The study was conducted with coordination led by Dr. Erik A. KARLSSON, Head of the Virology Unit. The objective of the research is to evaluate environmental surveillance methods for detecting infectious disease emergences or outbreaks in live animal markets in Cambodia. This project intends to scale up efforts in Cambodia, further develop the novel technologies employed, understand how the data is used for action and reporting The Platform has played a key role in supervising and conducting sequencing using Oxford Nanopore and Illumina technologies.

We anticipate performing metagenomics sequencing for 480 samples collected from live bird markets. To obtain an optimal sequencing protocol, preliminary experiments were performed on 25 samples with multiple conditions and sequencing platforms. The analysis of this data was completed, however, discussions among investigators to move forward are still ongoing.

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

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

The study has been carried-out in collaboration with the Virology Unit with Dr. Janin Nouhin as the Principal Investigator. In 2023, a total of 3 NGS runs were carried-out for the present study.

The main objective of the study is to describe and characterize human papillomavirus (HPV) diversity circulating in HIV-infected women in Cambodia using NGS approach. Secondarily, we will assess the associations between variants in HPV E6 and E7 coding genes and cervical intraepithelial neoplasia (CIN) status. The Sequencing Platform has been involved in performing HPV whole genome sequencing using rolling-circle amplification metagenomics approach and Illumina MiSeq technology. A total of 60 women co-infected with HIV and HPV were included in the study. The sequencing activities were completed for all included samples (20 samples in 2022 and 40 samples in 2023). Preliminary analysis indicated a poor outcome of metagenomic sequencing. As a result, 32 out of 60 samples had more than 10 reads of HPV sequence. To improve this result, we conducted additionnal sequencing run on 16 high-risk HPV samples using enrichment method, Twist CVRP Panel. However, the sequencing outcomes were not significantly improved. The bioinformatic and statical analysis were conducted using the outputs of metagenomics sequencing.

Data analysis is ongoing, and a publication is expected by end of 2024.

Research Project Name	Human papillomavirus E6 and E7 coding gene variations and their possible association with the occurrence of cervical intraepithelial neoplasia.
Funding	IPC Internal Funding
Project duration	2022 – 2023

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

5.4.2.2 User fee services

The Sequencing Mini-Platform provided fee-based user services for IPC and as well as external researchers.

- **Antimalarial resistance markers (*Pfk13*, *Pfcrt*, *Pfmdr1*, *Pfcytb*) in *Plasmodium falciparum* (Malaria Research Unit)**: The study emphasized the importance of genetic surveillance to monitor the spread of known molecular markers associated with antimalarial drug resistance in the Great Mekong Sub Region. The platform provided service for deep amplicon sequencing of antimalarial resistance markers (*Pfk13*, *Pfcrt*, *Pfmdr1*, and *Pfcytb*) of *Plasmodium falciparum.* All wet-lab procedures were carried-out within the Malaria Research Unit. Following the completion of the library preparation, the platform provided support to the study by conducting one run of NGS using the Illumina MiSeq system (Figure 2B).
- **DUKE-NUS Medical School:** In 2024, the platform provided service on metagenomics sequencing to a collaborative project between the Virology Unit, IPC and DUKE-NUS Medical School. The samples included in the project were confirmed-human Coronaviruses from SARI patients collected in Cambodia.

5.4.3 Research Programs - Outlook for 2024

The Sequencing Mini-Platform envisions an important role as a cornerstone technical facility supporting a wide spectrum of research projects across IPC. To sustain human resource capacity, we recruited and trained one laboratory technician in 2024. To provide a better affordability to our internal researchers, IPC leadership contributed financial supports in maintenance and depreciation cost, resulting in an approximate 78% reduction in equipment cost. Despite these efforts, the platform remains underutilized, with the Virology Unit being its primary user. Consequently, we plan to merge the platforms into a single facility hosted by the Virology Unit, effective April 1, 2025. This restructuring aims to optimize resource utilization while maintain comprehensive support for NGS-related activities across IPC and external customers.

Meeting and Workshop

- Mr. Vireak Heang was awarded Global Scholar to join annual meeting of American Society for Virology (ASV) from June 24 to 28, 2024 at Great Columbus, Ohio, USA. He presented a poster presentation entitled "Environmental Sampling and Viral Metagenomics at High-Risk Human-Animal Interfaces In Cambodia"
- Mr. Vireak Heang attended the training "Arboviral Genomic Surveillance in a climate evolving world" at Singapore from July 03 to 09, 2024.

5.4.4 Outlook for upcoming 3 – 5 years

The integration of the Sequencing Platform into the Virology Unit will significantly enhance the Unit's NGS capacity, fostering advanced genomic research and surveillance. This strategic plan will not only streamline technical expertise and resources but also position the Virology Unit as a leading training hub at both national and regional levels. Despite being embedded within the Virology Unit, the Sequencing Platform will maintain its pivotal role as facility supporting a wide spectrum of research projects across IPC.

5.4.5 Publication in 2024

Note: *The name of authors from the Institut Pasteur du Cambodge are underlined*

1. **Spatiotemporal evolution and transmission dynamics of Alpha and Delta SARS-CoV-2 variants contributing to sequential outbreaks in Cambodia during 2021.**
   Su YCF, Zeller MA, Ou TP, Ma J, Pum L, Zhang R, Rath S, Heang V, Kol S, Lim R, Chea KL, Khun L, Heng L, Krang S, Raftery P, Kinzer MH, Ieng V, Kab V, Patel S, Sar B, Horm VS, Yann S, Auerswald H, Siegers JY, Troupin C, Boukli N, Vandelannoote K, Wong FY, Ng GGK, Chan M, Sorn S, Sengdoeurn Y, Heng S, Darapheak C, Savuth C, Khalakdina A, Ly S, Baril L, Spiegel A, Duong V, Ly S, Smith GJD, Karlsson EA.Commun Med (Lond). 2024 Nov 28;4(1):252. doi: 10.1038/s43856-024-00685-7.

2. **Air sampling accurately captures circulating zoonotic viral diversity emerging from poultry live-animal markets.**
   Cronin P, Siegers J, Heang V, Tok S, Sin S, Sievers B, Omondi V, Nuon S, Chhel K, Nouhin J, Chim V, Seng B, Hak M, San S, Tum S, Claes F, Firth C, Su Y, Smith G, Karlsson E.Res Sq [Preprint]. 2025 Feb 13:rs.3.rs-5682962. doi: 10.21203/rs.3.rs-5682962/v1. Preprint (Under review at *Nature Communications*)

6 Services Support

6.1 Grant Office

6.1.1 Functional Structure

In 2024, the Grants Office was established to support fundraising activities and research management at IPC. Mrs. Najet Hadhri was recruited as Grants Officer, starting remotely in January 2024 and moving to on-site work in July 2024.

After conducting a preliminary needs assessment and portfolio analysis, an action plan was developed to structure research support services across both pre-award and post-award phases. To address the priority of managing the needs of ongoing projects, the office has been also reinforced with the recruitment of an additional resource, prioritizing post-award qualifications to ensure effective management of IPC projects portfolio. In this regard, Mr. Rithy Try joined the Grants Office in November 2024 as Research Funding Assistant.

The Grants Office reports to the Director General of IPC and works in close collaboration with DAF units and services.

IPC Grants Office acts as a central support hub, streamlining proposal applications and grant management. Its primary goal is to alleviate the workload of researchers by ensuring effective coordination and communication with partners and funders (Figure 1).

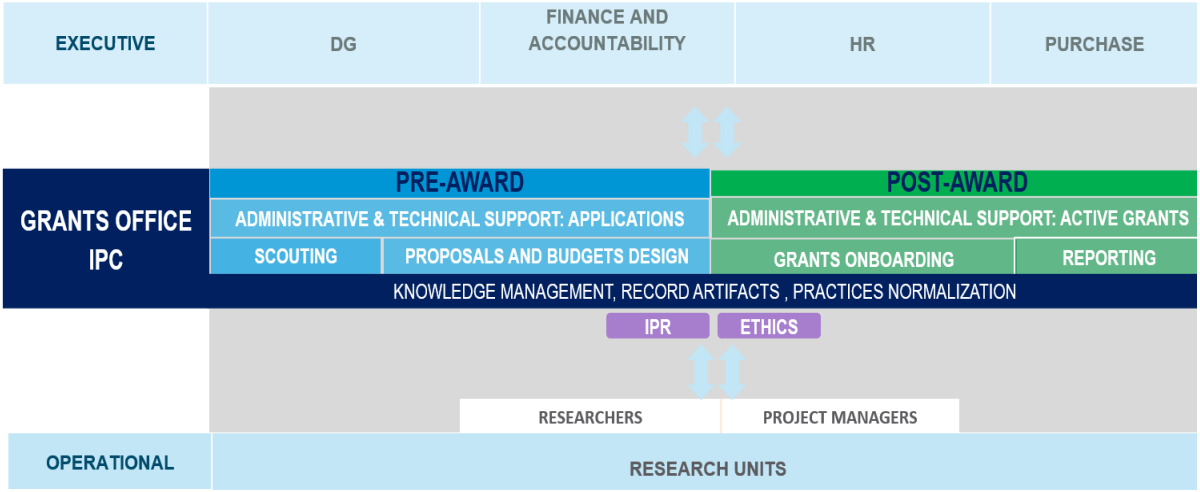


Figure 33. IPC Grants Office organization

IPC Grants Office is committed to establishing operational processes and workflows in order to:

- Provide guidance throughout the grant application process.
- Assist with grants onboarding, ensuring compliance with funding agencies and alignment with local requirements.
- Offer appropriate support during the reporting and renewal phases.
- Facilitate the identification and pursuit of funding opportunities to develop fundraising capacity and diversify resources.

6.1.2 Activities

In its first year, IPC's Grants Office has undertaken key tasks to refine both pre-award and post-award workflows. Significant progress has been made, with several milestones ahead.

Project Portfolio Management

A project directory has been established to track the institute's project portfolio. This directory integrates digital storage to improve accessibility, facilitate sharing, and support collaborative work across 60 listed projects (See Annex 2). Classification and registration rules have been set up to sustain accurate archiving and seamless grants artifacts retrieval.

Project Applications and Onboarding

This first year, GOIPC has provided assistance for applications and participation in 22 fundraising activities, including 6 proposals with leading roles within NIH, Wellcome Trust, and AFD. Actions benefited from punctual support during the preparation phase, including budget design and review, as well as handling administrative aspects related to the application process. Two coordinated initiatives (NIH, WT) were fully managed and coordinated with GOIPC support. Onboarding assistance has been provided for 10 new successful grant entries, comprising 5 coordinated projects (2 WT, 3 AFD) and 5 mono-beneficiary funds (NIH, CRDF, WHO, UNIDO, NHMRC) (Figure 2).

Collaboration and Contractual Guidance

In addition to managing the contractual aspects related to grant and consortium agreements, the IPC Grants Office has been involved in establishing collaborations with private entities for sponsored research projects and setting up specific terms for collaborative research agreements (Figure 2). Guidance on contractual aspects related to intellectual property and ethical considerations is provided whenever possible. To this end, provisional standard templates for MTAs, DTAs, NDAs, and MoUs have been made available.

Funding Opportunities and Scouting

Scouting of funding opportunities and matchmaking with IPC's needs and expertise have been performed. Four newsletters featuring 18 funding opportunities have been shared, in addition to direct dissemination to specific teams. Three proposal applications are accordingly scheduled in 2025.

Pre-Award	
Scouting	4
Application	12
Post-Award	
Awarding	15
Contract and renewal	14
Reporting	7
Other (CA, MTA, MoU)	
MTA, MoU	8
Collaborative Agreement	4
Consortium/Partnership Agreement	3

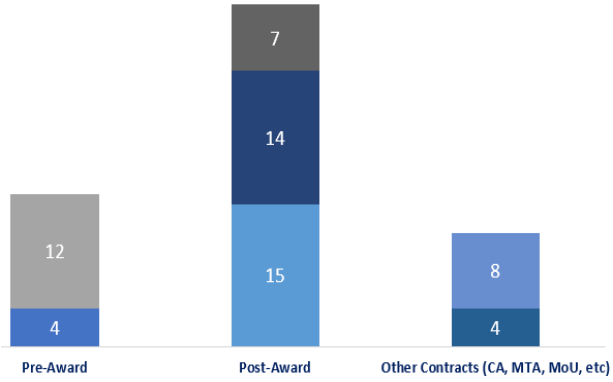


Figure 34. GO IPC performed tasks in 2024

6.1.3 IPC Grants Portfolio Highlights

IPC grants portfolio has 59 funded lines with active status, including 4 grants processed in 2024 with upcoming statuses in early 2025 (Table 1. IPC grants status 2024-2025).

The portfolio includes 51 project-based grants and 8 service contracts.

Table 12. IPC grants status 2024-2025.

Ongoing 2023	Start in 2024	Up coming Start in 2025	Total
25	31	5	61

Active funding in 2024 covers six key health areas that reflect the different units' specialties (Figure 3. Number of Projects Funded in 2024 by Research Theme).

There are **22** projects related to vector-borne and parasitic diseases, including 15 for malaria, 5 for dengue, 1 for chikungunya, and 1 for Japanese encephalitis. For respiratory viral diseases, there are 13 projects, including 10 for avian influenza, 1 for swine influenza, and 2 for COVID-19.

Public health and clinical research include 13 projects, with 3 for clinical research, 2 for clinical trials and HIV coinfection. There are 4 projects each for bacterial and mycobacterial infections (3 for TB and 1 for non-TB mycobacterial infections), and 4 for zoonotic and emerging diseases including CCHFV (1) and rabies (2). Environmental and food safety registered 2 funding entries each for food safety and wastewater/environmental surveillance.

In addition, 2024 has been marked by the entry of two important grants from WT for 2 new projects on One Health and Chikungunya (see Table 3**.** Grants Key entries for 2024-2025).

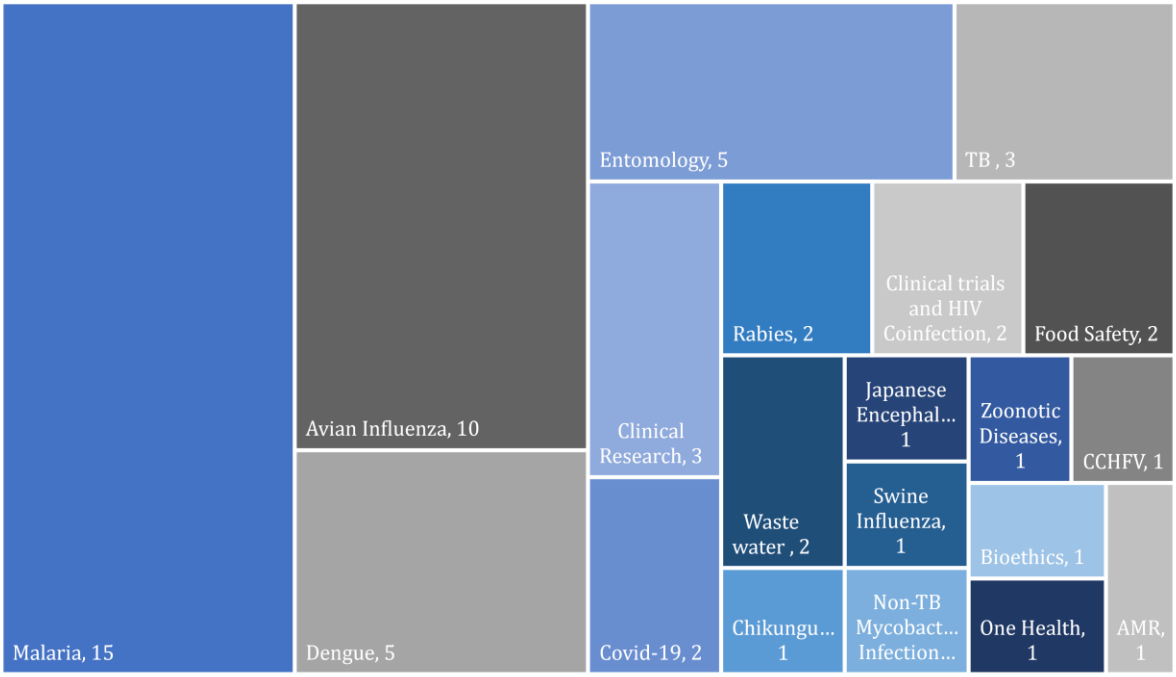


Figure 35. Number of Projects Funded in 2024 by Research Theme

Table 13. Grants Key entries for 2024-2025 with budget indication

	Acronym	Title	Start	End	Sponsor*
1.	VIRAGE	Vigilance Intégrée pour le Renforcement des Actions de Gestion et l'Élimination de la Rage au Cambodge AFD	1/04/2025	31/03/2027	AFD
2.	NTM	Nontuberculous mycobacterial (NTM) infections associated with climate change and major weather events: enhancing surveillance and mitigation strategies	01/03/2025	28/02/2028	CRDF

3.	RACSMEI	Risk Assessment of Community Spread of Multiple Endemic Infectious Disease pathogens in a One Health Perspective	01/01/2025	31/12/2030	WT
4.	ANRS 2025	Budget annuel du site ANRS du Cambodge 2025	01/01/2025	31/12/2025	ANRS
5.	PVCULT	Leveraging access to parasite natural diversity to identify Plasmodium vivax culture-adaptable strain	27/12/2024	30/11/2027	NIH
6.	CHIKV	Characterisation of the immunological mechanisms that drive chronic Chikungunya disease pathogenesis	15/11/2024	14/11/2030	WT
7.	RESPIRO	Aetiologic Agents of Community Acquired Pneumonia in South-East Asia	28/10/2024	27/01/2026	TTSH
8.	IMAGERIE	Un nouvel outil innovant au Cambodge: vers une révolution mondiale de l'image en sciences de la sante	18/07/2024	20/07/2025	French Embassy
9.	FAO 2024	Avian influenza surveillance activities in Cambodia, risk assessment of avian influenza, improving the utility of environmental sampling and field testing, and genetic analysis using novel sequencing techniques.	07/08/2024	31/12/2024	FAO
10.	CAPRED	Support to Institut Pasteur du Cambodge to set up two accredited testing laboratory services: Pesticides and-Antibiotics, and Heavy Metals Testing	22/04/2024	31/12/2026	DFAT-Australia
11.	AFRICAM	Preventing zoonotic diseases emergence	01/10/2024	30/06/2026	AFD/IRD
12.	SEA-ROADS	Approche régionale One Health pour la surveillance intégrée et interconnectée de la dengue urbaine en Asie du Sud-Est	01/10/2024	30/09/2027	ANRS

13.	DUKE NUS	Asia PGI Accelerating Pathogen Detection through Wastewater Surveillance	01/06/2024	30/11/2025	BMGF
14.	TRACKFLU	Tracking the spread of avian influenza viruses in live bird market networks	01/05/2024	30/04/2029	EC
15.	ALERTA	Assessing local entomological risks to support precision Public Health	01/05/2024	31/04/2025	IPP
16.	WHO TSA 2024	Provide laboratory testing for specimens and coordination of risk mapping	1/5/2024	31/12/2024	WHO
17.	NIAID-CEIRR	Influenza Surveillance, Risk Assessment, and Response: Swine Influenza Surveillance and Risk Assessment: Cambodia	01/01/2024	30/06/2024	Royal Veterinary
18.	ACIP CLIMPATHIC	Strategy for genomic surveillance of pathogens in wastewater	01/01/2024	31/12/2025	IP-ACIP
19.	METABODEN IRD	Impact of lipid metabolism on severe dengue: biomarkers and pathogenesis	01/01/2024	31/12/2026	ANR/IRD
20.	PREEMPT	Predicting the emergence and spread of drug resistant malaria parasites	01/01/2024	30/12/2027	NHMRC
21.	CCHVACIM	Crimean-Congo Hemorrhagic Fever Vaccine and Immunotherapy (CCHFV2)	01/01/2024	31/12/2027	EC

List of Funder Abbreviations and Countries

Acronym	Full Title	Country
AFD	Agence Française de Développement	France
ANR	Agence Nationale de la Recherche	France
ANRS	Agence Nationale de Recherches sur le Sida et les Hépatites Virales	France
CRDF	Civilian Research & Development Foundation	USA
DFAT	Department of Foreign Affairs and Trade - Australia	Australia
EC	European Commission	-
FAO	Food and Agriculture Organization of the United Nations	-
BMGF	Bill & Melinda Gates Foundation	USA
IP	Institut Pasteur, Paris	France
IP-ACIP	Institut Pasteur - Actions Concertées Inter-Pasteuriennes	France
IP-PIU	Institut Pasteur – Pasteur International Joint unit	France
IRD	Institut de Recherche pour le Développement	France

NHMRC	National Health and Medical Research Council	Australia
NIH	National Institutes of Health	USA
Royal Veterinary	The Royal Veterinary College	UK
TTSH	Tan Tock Seng Hospital	Singapore
WT	Wellcome Trust	UK

6.1.4 Outlook for the Next 3–5 Years

IPC Grants Office Objectives for Next Year:

- Support newly established groups in securing funding (six applications).
- Enhance resource planning and management tools, including JetReport.
- Develop SOPs to standardize and streamline grants management processes.
- Identify and apply for additional international funds and investigate opportunities with regional funders and networks to strengthen IPC's international and regional positioning.

3-Year Goals:

- Cultivate specialization in both pre-award and post-award processes, including expertise in the management of technology transfer and contractual aspects for collaborative research.
- Contribute to fostering strategic international alliances to broaden funding prospects and diversify sources, with a particular emphasis on regional stakeholders.

5-Year Goals:

- Establish IPC Grants Office as a cornerstone in IPC's strategic research management, acting as a driving force to promote collaboration and elevate competitiveness, with an expansion in funding and networks.

6.2 Communication Service

6.2.1 Functional Structure

Since May 15, 2024, the Communications Service of IPC has been led by Alexandre BLAISE, who took over from Anne-Céline PRIGENT following her departure. This marks the first time IPC has had a **full-time dedicated** staff member overseeing communications. The department is focused on promoting IPC's activities both internally and externally, collaborating closely with research units to organize events and effectively disseminate information. The department is supported by Executive Secretary **Marea PHEAP** and **Syna OP**, who play vital roles in ensuring the smooth functioning of the team, integrating design tasks with communication efforts to support the service efficiently.

6.2.2 Activities

6.2.2.1. Communication & Press/Media

The Communications Service is responsible for managing IPC's press and media relations, ensuring that the public is well-informed about IPC's research and public health initiatives. This involves organizing interviews, facilitating journalist interactions with scientists, and drafting press releases. The team is dedicated to maintaining a strong presence in the media, effectively communicating IPC's impact on public health.

To keep track of IPC's scientific publications, the Communication Service employs several methods, such as reviewing **PubMed** and collecting information directly from **IPC scientists**. After gathering this information, the team creates a bibliography that includes various metrics like the **impact factor** of each article, the contribution of each scientist in the **author list**, and the **DOI**. Monthly, published articles are collected and shared internally with all scientists at IPC. Furthermore, some of these articles are simplified and shared on IPC's social media to make the information more accessible to the public.

6.2.2.2. Communication & Web

To maintain an up-to-date and dynamic online presence, the Communications Service continuously updates the IPC website. Efforts are focused on ensuring that the "IPC News" and "Job Offers" sections are regularly updated, making key information transparent and accessible to both researchers and the general public. Significant updates have also been made to the pages dedicated to IPC's research units, such as Epidemiology & Public Health and Medical & Veterinary Entomology. Additionally, sections on health services, including the Medical Biology Laboratory, Laboratory of Environment & Food Safety, and International Vaccination Service, have been refined to improve user accessibility and clarity.

The website continues to serve as an essential source of information, attracting more, in 2024, than **100,000 sessions** and **over 200,000 page views**, with a predominantly Cambodian audience (64.1%), followed by users from the USA (11.3%) and France (5.4%). Visitors access the website almost equally from mobile devices (51%) and desktops (49%), emphasizing the need for a responsive and user-friendly interface. In 2024, the Insitute also receives 21 **comments** on its **Google pages**, and dedicated Google pages have been launched for IPC's rabies prevention centers in Kampong Cham and Battambang. Each comment is reviewed by the relevant unit head to ensure a prompt and appropriate response.

6.2.2.3. Communication & Social Media

IPC has developed a strong digital presence through platforms like Facebook, LinkedIn, and its official website to ensure continuous engagement with its audience. At the end of 2024, Facebook remains the department's primary social media platform, with over **41,769 followers**, ranking second in the

Pasteur Network. Over the past year, IPC posted **191 contents**, gained **3,300 new followers**, and accumulated 1.18 million total views. Engagement has been high, with more than 5,500 conversations initiated via Messenger and an average response time of just under 6 hours and 35 minutes. The majority of IPC's audience on Facebook is Cambodian (93%), with a smaller international following, including 1.6% from the USA and 1.2% from France.

On LinkedIn, IPC has **2,586 followers**, ranking 14th in the Pasteur Network. This platform has seen steady growth, with **132 posts** and **1,100 new followers** in the past year. IPC's LinkedIn page recorded **151,000 views**, with **190 posts** reshared by partners, helping to increase the institution's visibility within professional networks. The audience on LinkedIn is more internationally diverse, with 23% from France, 4% from Cambodia, and 1.2% from Singapore.

6.2.2.4. Events Communication

The service plays a significant role in promoting key events and initiatives, fostering engagement and collaboration while highlighting IPC's impact on public health. More than **24 events** were supported by communication. Among the major events supported by IPC's communication team is **CESRI**, an international scientific committee initiative that empowers local researchers to tackle critical public health challenges. IPC has also worked closely with the Australian Embassy on the **CAPRED & CAPFISH** projects, which focus on sustainable economic growth and responsible fisheries in Cambodia. A key initiative in collaboration with the French Embassy is the **KAP Rabies** project, which aims to raise awareness, educate, and prevent rabies in vulnerable Cambodian communities. Finally, IPC hosted the **Avian Influenza Symposium** in collaboration with the Pasteur Network, bringing together experts to share insights, research, and strategies to combat avian influenza while enhancing global preparedness.

The team has also actively contributed to the communication efforts surrounding **the International Angkor Wat Half Marathon**, an event that promotes health and wellness. This year, **104 participants** joined the event, 13 finished the 21km, further highlighting our commitment to community engagement and health awareness.

Angkor Wat Half Marathon, 2024 edition.

6.2.2.5. Design & Communication material

Throughout the year, the Communications Service has created various design materials to support IPC's initiatives, including booklets for Rabies Day, an Avian Influenza booklet showcasing old and new

pictures of IPC, and a newcomer booklet for human resources to help new staff integrate smoothly. Other materials include logos for events, kakemono digital invitations, and backdrops for the Avian Influenza Symposium. Additionally, the team has created numerous infographics and social media posts to summarize and simplify scientific knowledge, making it more accessible to a broader audience.

6.2.2.6. Pasteur Network Communication group in Asia Pacific Region (APAC)

Initiated by IPC's Communications Service in collaboration with Pasteur Network focal points, a monthly meeting was launched for communicators across the **Pasteur Network in the APAC region** (9 institute in total). This platform allows participants to exchange tips, techniques, and learn from each other. The first and second meetings were led by Cambodia, and the third meeting focused on a **Canva software tutorial**, which was extended to other institutes in the network.

6.2.2.7. Satisfaction survey

The service has also implemented satisfaction surveys across different entities, including the Laboratory of Environment and Food Safety, Medical Biology Laboratory, and Rabies Prevention Centers in Phnom Penh, Battambang, and Kampong Cham. Since June 2024, over **2,500 responses** have been received, with a **satisfaction rate of 91.3%** and a **Net Promoter Score (NPS) of 61.3**. Monthly reports are shared with unit heads to ensure continuous improvement. In addition to regular surveys, IPC occasionally develops specialized surveys, such as an annual IT survey and gender survey, to address specific needs.

6.2.3 Outlook for the Next 3–5 Years

6.2.3.1. Three-Year Plan

IPC's website will undergo a complete redesign to enhance user experience, visual appeal, and accessibility. This will involve collaboration with web designers, user experience research, and content migration. Training programs in design and communication will be developed for secretarial and executive staff, based on surveys and feedback, with external trainers or e-learning modules incorporated where necessary.

A specialized communication strategy within research units will streamline information sharing. Dedicated communication groups will be created, using standardized templates. To reach younger audiences, IPC will launch an official TikTok account, producing engaging short-form videos on public health topics. Budgeting for video production equipment and editing software will ensure high-quality content. Internally, a staff satisfaction survey will assess communication effectiveness, and efforts will be made to improve the visibility of IPC's scientific publications for broader dissemination.

6.2.3.2. Five-Year Vision

By 2029, IPC aims to have significantly enhanced its digital presence, solidifying its position as a leading public health communication entity within Cambodia and the broader Pasteur Network. The department will focus on expanding its reach across digital platforms, developing engagement-driven content, and ensuring that IPC remains at the forefront of scientific communication. Increased collaboration with international institutions on social media will be a priority, allowing IPC to extend its influence and impact on global public health discussions. This will involve actively participating in social media conversations, tagging relevant organizations, and increasing participation in international webinars and conferences to strengthen IPC's presence and contributions to global health.

7 Scientific Publications in 2024

Note: *The name of authors from the Institut Pasteur du Cambodge are underlined*

1. **A call to innovate Antarctic avian influenza surveillance.**
   Wille M, Dewar ML, Claes F, Thielen P, Karlsson EA.
   Trends Ecol Evol. 2024 Nov 29:S0169-5347(24)00278-7. doi: 10.1016/j.tree.2024.11.005.

2. **A genotyping array for the globally invasive vector mosquito, Aedes albopictus**
   L. V. Cosme, M. Corley, T. Johnson, D. W. Severson, G. Yan, X. Wang, N. Beebe, A. Maynard, M. Bonizzoni, A. Khorramnejad, A. Jesus Martins, J. B. Pereira Lima, L. E. Munstermann, S. N. Surendran, Chun-Hong Chen, K. Maringer, I. Wahid, S. Mukherjee, J. Xu, Michael C. Fontaine, E. L. Estallo, M. Stein, T. Livdahl, P. Y. Scaraffia, B. H. Carter, M.Mogi, N. Tuno, J. W. Mains, K. A. Medley, D. E. Bowles, Richard J. Gill, R. Eritja, R. González-Obando, H. T. T. Trang, S. Boyer, A. M. Abunyewa, K. Hackett, Tina Wu, J. Nguyễn, J. Shen, H. Zhao, J. E. Crawford, P. A. & Adalgisa Caccone.
   Parasites & Vectors volume 17, Article number: 106 (2024) Published: 04 March 2024 https://doi.org/10.1186/s13071-024-06158-z.

3. **Aedes unalom sp. nov. in Cambodia, a new Stegomyia species close to Aedes albopictus (Diptera, Culicidae)**
   M. Hide, P.O Maquart, S. Yean, K. Suor, K. Heng, S. Sen, C. Yim, S. Leng, A. Rakotonirina, D. Fontenille, S. Boyer
   Journal of Asia-Pacific Entomology, Volume 27, Issue 2, June 2024, 102233 https://doi.org/10.1016/j.aspen.2024.102233.

4. **Antibiotic resistance profiles of sentinel bacteria isolated from aquaculture in Cambodia.**
   Peng C, Moniroth S, Khy P, Chea S, Thanh C, Heng O, Sarter S, Cheng S, Caruso D.
   J Water Health. 2024 Jun;22(6):1033-1043. doi: 10.2166/wh.2024.101. Epub 2024 May 8. https://doi.org/10.2166/wh.2024.101.

5. **Balancing functions of regulatory T cells in mosquito-borne viral infections**
   S. Sann, M. Kleinewietfeld, T. Cantaert
   Emerging Microbes & Infections 2024, https://doi.org/10.1080/22221751.2024.2304061.

6. **Bayesian modeling of post-vaccination serological data suggests that yearly vaccination of dog aged <2 years old is efficient to stop rabies circulation in Cambodia.**
   Auerswald H, Guillebaud J, Durand B, Le Vu M, Sorn S, In S, Pov V, Davun H, Duong V, Ly S, Dussart P, Chevalier V.
   PLoS Negl Trop Dis. 2024 Apr 18;18(4):e0012089. doi: 10.1371/journal.pntd.0012089. eCollection 2024 Apr. PMID: 38635851

7. **Bayesian accuracy estimates for diagnostic tests to detect tuberculosis in captive sun bears (Helarctos malayanus) and Asiatic black bears (Ursus thibetanus) in Cambodia and Vietnam.**
   Officer K, Arango-Sabogal JC, Dufour S, Lyashchenko KP, Cracknell J, Thomson S, Cheng S, Warren K, Jackson
   B. PLoS One. 2024 Nov 13;19(11):e0313007. doi: 10.1371/journal.pone.0313007. eCollection 2024.

8. **Challenges for ticks and tick-borne diseases research in Southeast Asia: Insight from the first international symposium in Cambodia**

S. Yean, D. B. Prasetyo, S. Marcombe, U. K. Hadi, A.R. Kazim, S. Tiawsirisup, V. Duc Chinh, K. Matsuno, V. Lun Low, S. Bonnet, N. Boulanger, T. Tsan-Yuk Lam, M. Yazid Abdad, V. Herbreteau, J.M. Chavatte, S. Sum,Theary Ren, A. Sakuntabhai, P.O Maquart, A. Rakotonirina,S. Boyer.
PLoS Negl Trop Dis PMCID: PMC11236135 DOI: 10.1371/journal.pntd.0012269

9. **Challenges of rabies surveillance in Madagascar based on a mixed method survey amongst veterinary health officers.**
   Dreyfus A, Volasoa MH, Guis H, Razafindraibe NP, Razafindramparany MH, Arivony NL, Rakotondrabe N, Andriamananjara MA, Dussart P, Kassie D, Lacoste V, Andriamandimby SF.
   Front Vet Sci. 2024 Feb 28;11:1270547. doi: 10.3389/fvets.2024.1270547

10. **Climate-driven models of leptospirosis dynamics in tropical islands from three oceanic basins.**
    Douchet L, Menkes C, Herbreteau V, Larrieu J, Bador M, Goarant C, Mangeas M.
    PLoS Negl Trop Dis. 2024 Apr 25;18(4):e0011717. doi: 10.1371/journal.pntd.0011717

11. **Colistin resistance in ESBL- and Carbapenemase-Producing Escherichia coli and Klebsiella pneumoniae clinical isolates in Cambodia.**
    Hide M, Meng S, Cheng S, Bañuls AL, Ky S, Chantana Y, Laurent D, Delvallez G.
    J Glob Antimicrob Resist. 2024 Jul 12:S2213-7165(24)00134-6. doi: 10.1016/j.jgar.2024.06.017.

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    PLoS Negl Trop Dis. 2024 Jan 29;18(1):e0011926. doi: 10.1371/journal.pntd.0011926

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    Pepey A, Souris M, Kim S, Obadia T, Chy S, Ea M, Ouk S, Remoue F, Sovannaroth S, Mueller I, Witkowski B, Vantaux A.
    Malar J. 2024 Mar 12;23(1):75. doi: 10.1186/s12936-024-04890-6.

14. **Copy Number Variations of Plasmodium vivax DBP1, EBP/DBP2, and RBP2b in Duffy-positive and Duffy-negative Ethiopians.**
    Pestana K, Ford A, Rama R, Abagero B, Kepple D, Tomida J, Popovici J, Yewhalaw D, Lo E.
    J Infect Dis. 2024 Aug 5:jiae388. doi: 10.1093/infdis/jiae388.

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    d'Elbée M, Harker M, Mafirakureva N, Nanfuka M, Huyen Ton Nu Nguyet M, Taguebue JV, Moh R, Khosa C, Mustapha A, Mwanga-Amumpere J, Borand L, Nolna SK, Komena E, Cumbe S, Mugisha J, Natukunda N, Mao TE, Wittwer J, Bénard A, Bernard T, Sohn H, Bonnet M, Wobudeya E, Marcy O, Dodd PJ; TB-Speed Health Economics Study Group.
    EClinicalMedicine. 2024 Mar 21;70:102528. doi: 10.1016/j.eclinm.2024.102528.

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D.Kania, J. Nouhin, K. Bolloré, R. Njouom, T. d'A. Toni, A. Issiaka Maiga, C. Toure-Kane, N. Ngo-Giang-Huong, A. Dagnra, D. Hoang Chuong, F. Lunel-Fabiani, J. Castera-Guy, P. A. Rubbo, A. Pisoni, J.C Plantier, E. Tuaillon
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Wobudeya E, Nanfuka M, Ton Nu Nguyet MH, Taguebue JV, Moh R, Breton G, Khosa C, Borand L, Mwanga-Amumpaire J, Mustapha A, Nolna SK, Komena E, Mugisha JR, Natukunda N, Dim B, de Lauzanne A, Cumbe S, Balestre E, Poublan J, Lounnas M, Ngu E, Joshi B, Norval PY, Terquiem EL, Turyahabwe S, Foray L, Sidibé S, Albert KK, Manhiça I, Sekadde M, Detjen A, Verkuijl S, Mao TE, Orne-Gliemann J, Bonnet M, Marcy O; TB-Speed Decentralisation study group.
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de Block T, De Baetselier I, Van den Bossche D, Abdellati S, Gestels Z, Laumen JGE, Van Dijck C, Vanbaelen T, Claes N, Vandelannoote K, Kenyon C, Harrison O, Santhini Manoharan-Basil S.
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B. Fortune Melingui, J. Basant, J. voisin Taguebue, D. Mbang Massom, E. Leroy Terquem, P. Yves Norval, A. Salomao, B. Dim, C. Eap Tek, L. Borand, C. Khosa, R. Moh, J. Mwanga-Amumpere, M. Tan Eang, I. Manhiça, A. Mustapha, E. Balestre, S. Beneteau, E. Wobudeya, O.r Marcy, J. Orne-Gliemann, M. Bonnet, on behalf of the TB-Speed Decentralization Study Group
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J. D Ratcliff , B. Merritt , H. Gooden , J. Y Siegers, A. Srikanth, S. Yann, S. Kol, S. Sin, S. Tok, E. A Karlsson, P. M Thielen
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Sann S, Heng B, Vo HTM, Arroyo Hornero R, Lay S, Sorn S, Ken S, Ou TP, Laurent D, Yay C, Ly S, Dussart P, Duong V, Sakuntabhai A, Kleinewietfeld M, Cantaert T.
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S. Iv, C.Nguon, P. Kong, T. Sieng, S. Srun, C. Christiansen-Jucht, C. Kul, T. Lorn, Sophy Chy, J. Popovici, A. Vantaux, B. Witkows, A. Berry, P.Piola, C. Flaman d.
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33. **Metabolic Resistance and Not Voltage-Gated Sodium Channel Gene Mutation Is Associated with Pyrethroid Resistance of *Aedes albopictus* (Skuse, 1894) from Cambodia.**
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Insects. 2024 May 15; https://doi.org/10.3390/insects15050358.

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C. L. Jeffries, L. M Tantely, P. Kadriaj, M.S C Blagrove, L. Lytra, J. Orsborne, H. M. Al-Amin, Abdul Rahim Mohammed, M. Shafiul Alam, R. Girod, Y. A Afrane, S. Bino, V. Robert, S. Boyer, M. Baylis, E. Velo, G. L Hughes, T.Walker
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Gyamfi E, Delvallez G, Cheng S, Meng S, Oeurn K, Sam C, Kerleguer A, Guillard B, Bañuls AL, Hide M. - PLoS Negl Trop Dis. 2024 Nov 14;18(11):e0012652. doi: 10.1371/journal.pntd.0012652.

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8 Annexes

8.1 List of Acronyms

A*STAR	Agency for Science, Technology and Research, in Singapore
AB	Antibody
ACE	Australian Center for Education
ACIP	Actions Concertées Inter-Pasteuriennes
ACREME	Australian Centre of Research Excellence in Malaria Elimination
ADE	Antibody Dependent Enhancement
AFB	Acide-Fast Bacilli
AFD	Agence Française pour le Développement (French Development Agency)
AFRIMS	Armed Forces Research Institute of the Medical Sciences
AHF	AIDS Healthcare Foundation
AI	Artificial Intelligence
AIDS	Acquired Immunodeficiency Syndrome
AIRD	Agence Inter-Etablissements de Recherche pour le Développement
AIV	Avian Influenza Virus
ALERTA	Assessing local entomological risks to support precision Public Health
AMR	Antimicrobial Resistance
ANR	Agence Nationale de la Recherche
ANRS	Agence Nationale de Recherche sur le SIDA (National Agency for AIDS Research)
ANRS-MIE	Agence Nationale de Recherche sur le SIDA, les Hépatites et les Maladies Infectieuses et Emergentes (French National Agency for AIDS and Hepatitis Research)
ANU	Australian National University
APAC	Asia Pacific
ARCAHE	Antibiotic resistance at the human/animal/environment interface
ART	Antiretroviral Therapy
ASEAN	The Association of Southeast Asian Nations
Asia-PGI	Asia Pathogen Genomics Initiative
ASV	American Society for Virology
ASFV	African swine fever virus
AVSF	Agronomes et Vétérinaires Sans Frontières
BAAR	Bacteriology and Antibiotic Resistance
BAIA	Bioinformatics and AI Applications
BCOMING	Biodiversity Conservation to Mitigate the Risks of Emerging Infectious Diseases
BCR	B-Cell Receptor
BIOSEC	Biosecurity
BIOSPC	Bio Sorbonne Pasris Cité
BMGF	Bill & Melinda Gates Foundation
BMT	Bone Marrow Transplant
BSC	Biological Safety Cabinet
BSL3	Biosafety Level 3
BSLII	Biosafety Level 2
BTRP	Biological Threat Reduction Program
CADT	Cambodian Academy of Digital Technology

CANARIES	Consortium of Animal Market Networks to Assess Risk of Emerging Infectious Diseases through Enhanced Surveillance
CAP	Community-Acquired Pneumonia
CAPFISH	Capture Post-Harvest Fisheries
CAPRED	Cambodia Australia Partnership for Resilient Economic Development
CBRN	Chemical, Biological, Radiological and Nuclear
CCBR	Cambodian Committee for TB Research
CCHFV	Crimean-Congo Haemorrhagic Fever Virus
CCHFVACIM	Crimean-Congo Haemorrhagic Fever Vaccine and Immunotherapy
CDC	Center for Disease Control and Prevention in Cambodia
CEIRR	Centers for Excellence in Influenza Research and Response
CENAT	National Center for Tuberculosis and Leprosy Control
CFO	Chief Financial Officer
CHAI	Clinton Health Access Initiative
ChatGPT	Chat Generative Pre-Trained Transformer
CHIKV	Chikungunya Virus
CILM	Centre d'Infectiologie Lao Christophe Mérieux
CIN	Cervical Intraepithelial Neoplasia
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement (French Agricultural Research Centre for International Development)
CISED	Comprehensive Integrated Surveillance and Early Detection System for Dengue
CITE-seq	Cellular Indexing of Transcriptomes and Epitopes by Sequencing
CJFMBRH	Cambodia Japan Friendship Mongkul Borey Referral Hospital
CNM	National Center for Parasitology, Entomology, and Malaria Control (Cambodia)
CNRS	National Center for Scientific Research / Centre national de la recherche scientifique
COFRAC	French Accreditation Committee (Comité Français d'Accréditation)
COI	Cytochrome C Oxidase Subunit 1
COPD	Chronic Obstructive Pulmonary Disease
Co-PI	Co-Principal Investigator
COVID-19	Coronavirus Disease 2019
CPC	Centre Pasteur du Cameroun
CPE	Carbapenemase-Producing Enterobacteriaceae
CPR	Cardiopulmonary Resuscitation
CRAB	Carbapenem-Resistant A. Baumannii
CRDF	Civilian Research and Development Foundation
CRE	Carbapenem-Resistant Enterobacteriaceae
CREID	Centers for Research in Infectious Diseases
CRPA	Pseudomonas Aeruginosa Carbapenem Resistant
CVRP	Comprehensive Viral Research Panel
CWRU	Case Western Reserve University (USA)
DAA	Direct Acting Antiviral
DAF	Directeur administratif et financier
DATURA	Determination of Adequate Tuberculosis Regimen in Adults and Adolescents
DBP	Duffy Binding Protein
DC	Distribution Centre
DEMELE-JEV	Diagnostics and surveillance of acute meningo-encephalitis among children in Cambodia with a focus on Japanese Encephalitis Virus

DENTHOM	Study of Dengue and Dengue-Like Illnesses in Kampong Thom Province, Cambodia
DENV	Dengue Viruses
DFAT	Department of Foreign Affairs and Trade - Australia
DHF	Dengue Hemorrhagic Fever
DIR	Director / Direction
DKSH	DiethelmKellerSiberHegner
DNA	Deoxyribonucleic Acid
DOI	Digital Object Indentifier
DSMB	Data and Safety Monitoring Board
DTA	Deep Trade Agreement
ECACC	European Collection of Authenticated Cell Cultures
EBP	Evidence-based practice
EC	European Commission
ECOMORE	Economic Development, Ecosystem Modifications and Emerging Infectious Diseases Risk Evaluation
ECTS	European Credit Transfer and Accumulation System
EDE	E-Dimer Epitope
eDNA	Environmental Deoxyribonucleic acid
EEHV	Elephant Endotheliotropic Herpesvirus
EFEO	École francaise d'Extreme Orient
EHA	EcoHealth Alliance
EIC	Education, Information and Communication
ELISA	Enzyme-linked Immunosorbent Assay
EPHU	Epidemiology and Public Health Unit
ERIG	Equine Rabies Immunoglobulins
ES	Environmental Sampling
ESBL	Extended Spectrum Beta Lactamase
ETI	International Technical Expert
EU	The European Union
EWARS	Early Warning, Alert and Response System
FAO	Food and Agriculture Organization of the United Nations
FAVNT	Fluorescent Antibody Virus Neutralization Test
Fc	Fragment Crystallizable
FC	Food Contact
FCS	Food Contact Surface
FEF	French Ministry of Europe and Foreign Affaires through the Solidarity Funds for Innovative Projects
FEF-R	Fond Equipe France Rapide, Paris, France
FL	Fusion Loop
FORT	Foodborne Outbreak Response Team
FSPI	Solidarity Fund for Innovative Project
FSPI-R	French Ministry of Europe and Foreign Affairs through the Solidarity Funds for Innovative Projects
G6PD	Glucose 6-Phosphate Dehydrogenase
GCRF	UK Global Challenges Research Fund
GDAHP	General Directorate of Animal Health and Production
GDPR	General Data Protection Regulation
GF-AAS	Graphite Furnace Atomic Absorption Spectroscopy

GIS	Geographic Information System
GIZ	German Agency for International Cooperation
GMS	Greater Mekong Subregion
GO	Grants Office
GPS	Global Positioning System
HA	Hemagluttinin
HBcrAg	Hepatitis B correlated antigen
HBV	Hepatitis B Virus
HCMC	Ho Chi Minh City
HCV	Hepatitis C Virus
HDR	Habilitation à Diriger des Recherches
H.E	His/Her Excellency
Hep A	Hepatitis A Virus
HEPEDIAC	Study of DAA Treatment for Children and Adolescents with Active HCV Infection in Cambodia
HEV	Hepatitis E Virus
HHMI	Howard Hughes Medical Institute Inc
HIB	Haemophilus Influenzae Type B
HIV	Human Immunodeficiency Virus
HIVDR	Human Immunodeficiency Virus Drug Resistance
HPAI	Highly Pathogenic Avian Influenza
HPC	High Performance Computing
HPV	Human Papillomavirus
HR	Human Resource
hrHPV	High-risk Human Papillomavirus
HSD	Health and Social Development
HSeQM	Hygiene, Security, Environment, Quality and Maintenance
IAEA	International Atomic Energy Agency
IAS	International Accreditation Service
IAV	Swine Influenza A Virus
ICEMR	International Center of Excellence in Malaria Research
ICER	International Center for Excellency in Research
ICTMM	International Congress for Tropical Medicine and Malaria
ID	Intradermal Injections
IDE	International Development Enterprises
iDES	Integrated Drug Efficacy Surveillance
IDEES	Identités et Différenciations de l'Environnement des Espaces et des Sociétés
IEC	International Electrotechnical Commission
IFI	Invasive Fungal Infections
IFN	Interferons
IgG	Immunoglobulin G
IGHB	Immunoglobulin Hepatitis B
IGRA	Interferon-Gamma Release Assays
ILI	Influenza-Like Illness
IM	Intramuscular
INSERM	Institut National de la Santé et de la Recherche Médicale (France)
IP	Institut Pasteur, Paris
IP-ACIP	Institut Pasteur – Action Concertées Inter-Pasteuriennes

IPC	Institut Pasteur du Cambodge
IP HCMC	Institut Pasteur Ho Chi Minh City
IPIN	Institut Pasteur International Network
IPL	Institut Pasteur du Laos
IPNC	Institut Pasteur New Caledonia
IPP	Institut Pasteur in Paris
IP-PIU	Institut Pasteur – Pasteur International Joint Unit
IPTf	Intermittent Preventive Treatment for Forest Goers
IPR	Intellectual Property Rights
IQC	Internal Quality Control
IRASEC	Institut de recherche sur l'Asie du sud-est contemporaine
IRD	Institut de Recherche pour le Développement (Institute for Research and Development- France)
IRIM	International Research Institute for Manufacturing
IRIS	Immune Reconstitution Inflammatory Syndrome
ISO	International Organization for Standardization
ISVEE17	17th International Symposium on Veterinary Epidemiology and Economics
IT	Information Technology
ITC	Institute of Technology of Cambodia
IVC	International Vaccination Center
IVI	International Vaccine Initiative
JEV	Japanese Encephalitis Virus
JRP	Joint Research Program
KAP	Knowledge, Attitudes and Practices
KBH	Kantha Bopha Hospital
KHEOBS	Khmer Earth Observation Laboratory
KOICA	Korea International Cooperation Agency
KOM	Kick Off Meeting
LAM	Lipoarabinomannan
LBM	Live Bird Market
LEFS	Laboratory of Environment and Food Safety
LFRD	Lycée Français René Descartes
LIMS	Laboratory Information Management System
LILAC-TB	Lowering InterLeukin-1 receptor antagonist concentrations after TB treatment
LIPS	Luciferase Immunoprecipitation System
LIS	Laboratory Information System
LLMs	Large Language Models
LMICs	Low-and-Middle-Income Countries
LMI-DRISAOH	Laboratoire Mixte International - Drug Resistance in Southeast Asia: A One Health Approach to Tackle AMR Spread
LMVR	Laboratory of Malaria and Vector Research
LPAI	Low Pathogenic Avian Influenza
LSHTM	London School of Hygiene and Tropical Medicine UK
LTBI	Latent Tuberculosis Infection
mAbs	Monoclonal Antibodies
MAE	Master of Applied Epidemiology
MAFF	Ministry of Agriculture, Forestry, and Fisheries (Cambodia)
MAT	Microscopic Agglutination Test

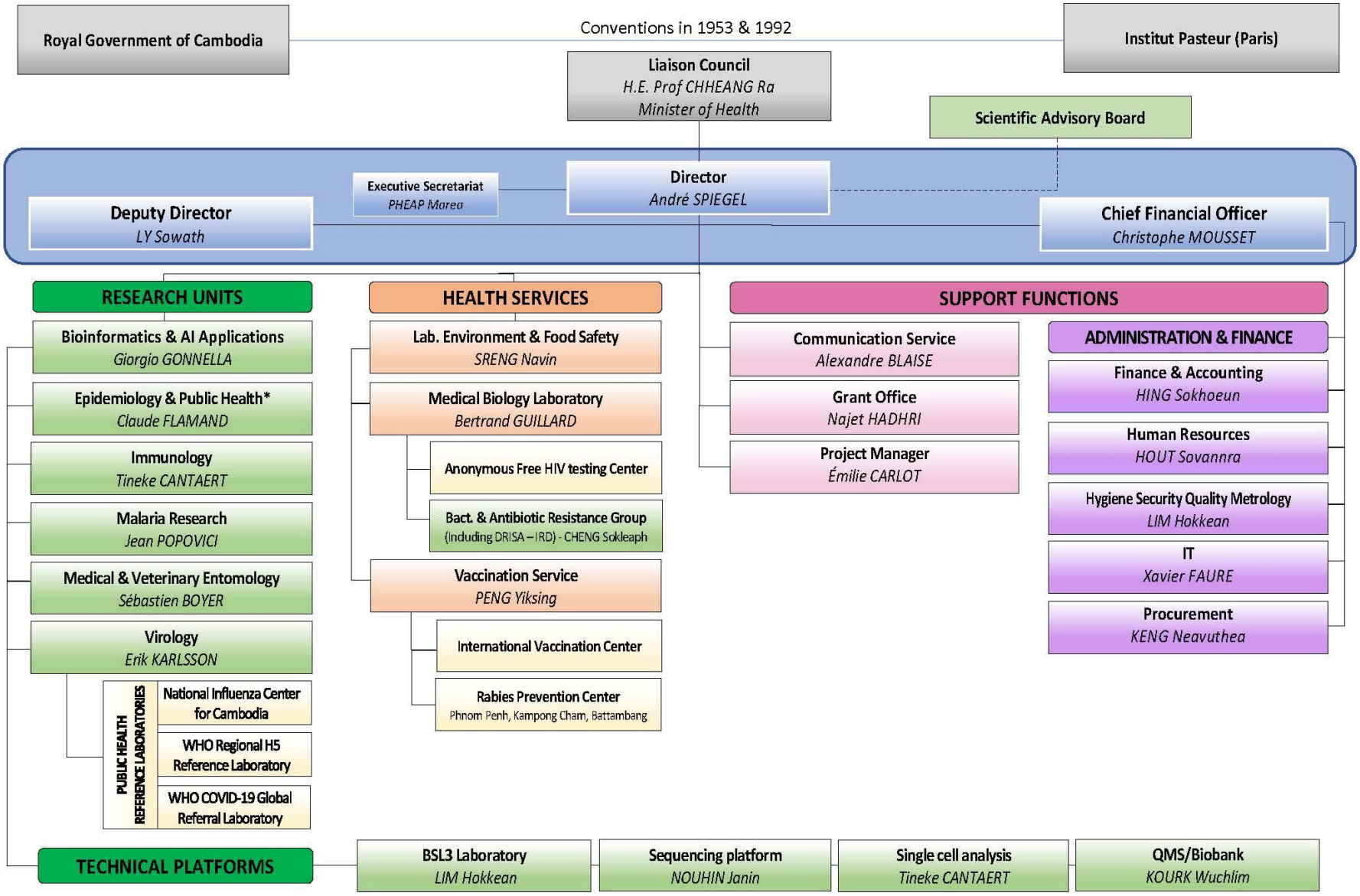
MBL	Medical Biology Laboratory
MDR-E	Muti-Drug-Resistant Enterobacteriaceae
MEAE	Ministère de l'Europe et des Affaires Etrangères (French Ministry for Europe and Foreign Affairs)
MIA	Multiplex Immunoassay
MIE	Maladies Infectieuses Émergentes
MIVEGEC	Maladies Infectieuses et Vecteurs Écologie, Génétiques, Évolution et Contrôle (IRD, France)
MMV	Medicines for Malaria Venture
MoE	Ministry of Environment
MoH	Ministry of Health of Cambodia
MoUs	Memorandum of Understanding
Mpox	Monkeypox (Viral Disease)
MRSA	Methicillin Resistant Staphylococcus Aureus
MRU	Malaria Research Unit
MSC	Masters of Sciences
MTA	Material Transfer Agreement
MTB	Mycobacterium Tuberculosis
MTBC	Mycobacterium Tuberculosis Complex
MTCT	Mother to child transmission
N_2	Nitrogen
NAAT EQA	Nucleic Acid Amplification Testing External Quality Assurance
NABM	Nomenclature des Actes de Biologie Médicale
NAbs	Neutralizing Antibodies
NACW	National Authority for Chemical Weapons
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)
NCLE	The National Center for Laboratory and Epidemiology
NDAs	Non-disclosure Agreement
NDCP	National Dengue Control Program
NGO	Non-Governmental Organization
NGS	Next Generation Sequencing
NHMRC	National Health and Medical Research Council
NIAID	National Institute of Allergy and Infectious Diseases
NIC	National Influenza Center
NIH	National Institutes of Health
NIHE	National Institute of Hygiene and Epidemiology
NIMPE	National Institute of Malariology, Parasitology and Entomology
NIP	National Immunization Program
NIPH	National Institute for Public Health
NK Cell	Natural Killer Cell
NMCHC	National Maternal Child Health Center
NMCP	National Malaria Control Program
NPJ Vaccines	Nature Partner Journals Vaccines
NPS	Net Promoter Score
NSSF	National Social Security Fund

NTM	Nontuberculous Mycobacterial
NTU	Nanyang Technological University
NUS	National University of Singapore
ODK	Open Data Kit
OH SEA	One Health in Practice in Southeast Asia
ONT	Oxford Nanopore Technologies
OPTICAM	Optimizing Latent Tuberculosis Treatment Initiation in Cambodia among People Living with HIV
OUCRU	Oxford University Clinical Research
PACAI	Preventing Avian Influenza in the Pacific
PAP	Peripartum antiviral prophylaxis
PAPIBI	Pasteur Asia-Pacific International Bioinformatics Initiative
PCR	Polymerase Chain Reaction
PEACH	Pathogens Exposure from Aquifers: A Cambodian Health interdisciplinary case study
PEP	Post Exposure Prophylaxis
Pfcrt	Plasmodium falciparum chloroquine resistance transporter
Pfcytb	Plasmodium falciparum cytochrome b
PfD	Partners for Development
Pfk13	Plasmodium falciparum Kelch 13
Pfmdr1	Plasmodium falciparum multidrug resistance 1
PhD	Doctor of Philosophy (degree)
PHD	Provincial Health Department
PHEIC	Public Health Emergency of International Concern
PICREID	Pasteur Institute – Center for Research for Emerging Infectious Diseases
PICTs	Pacific Island Countries and Territories
PIU	Pasteur International Research Unit
PIV	Parainfluenza Virus
PLHIV	People Living with HIV
PN	Pasteur Network
PNG	Papua New Guinea
PNGIMR	Papua New Guinea Institute of Medical Research
PP	Phnom Penh
PPR	Epidemic Preparedness and Response
PREZODE	Preventing Zoonotic Disease Emergence
PTLD	Post-Tuberculosis Lung Disease
PTWRC	Tamao Zoological Park and Wildlife Rescue Center
Pv	Plasmodium vivax
PVCULT	Plasmodium vivax culture-adaptable strain
PvDBP	Plasmodium vivax Duffy Binding Protein
QR	Quick Response
RABV	Rabies Virus
RACSMEI	Risk Assessment of Community Spread of Multiple Endemic Infectious Disease pathogens in a One Health Perspective
RBP2b	Reticulocyte-binding protein 2b
RDT	Rapid Diagnostic Test
RIF	Resistance to Rifampicin
RIG	Rabies Immunoglobulin

RITM	Research Institute of Tropical Medicine in Manila
RNA	Ribonucleic Acid
RPC	Rabies Prevention Centers
RSV	Respiratory Syncytial Virus
RUA	Royal University of Agriculture
RVC	Royal Veterinary College
SAB	Scientific Advisory Board
SARI	Severe Acute Respiratory Illness
SARS-CoV-2	Severe Acute Respiratory Illness, COVID-19- 2
scRNAseq	Single Cell RNA Sequencing
SDSV	Structure et Dynamique des Systèmes Vivants
SEA TICKEY	Southeast Asia Tick Identification Key
SFTSV	Server Fever with Thrombocytopenia Syndrome Virus
SOP	Standard Operating Procedures
SRPRH	Svay Rieng Provincial Referral Hospital
STD	Sexually Transmitted Diseases
STIs	Sexually Transmitted Infections
SWOT	Strengths Weaknesses Opportunities and Threats
TB	Tuberculosis
TBEV	Tick-Borne Encephalitis Virus
TBV	Transmission-blocking vaccine developent
TCR	T Cell Receptor
TEMVS	Targeted Enriched Viral Metagenomic Sequencing
TEMRA	Terminal Effector Memory T Cell
TES	Therapeutic Efficacy Studies
TM	Technical Manager
TPT	Tuberculosis Preventive Treatment
TRACKFLU	Tracking the spred of avian influenza viruses in live bird market networks
TST	Tuberculin Skin Tests
TTSH	Tan Tock Seng Hospital
TWG	Technical Working Group
UGA	University of Georgia
UHS	University of Health Sciences
UMB	University of Maryland, Baltimore
UMD	University of Maryland
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNICEF	United Nations International Children's Emergency Fund
UNIDO	United Nations Industrial Development Organization
UP	University of Puthisastra
UPS	Université Paris-Saclay in France
URCA	Université de Reims Champagne-Ardenne
USA	United States of America
USAID	United State Agency for International Development
USF	University of South Florida in USA
USTH	University of Science and Technology of Hanoi
VCCT	Voluntary Confidential Counselling and Testing for HIV
VCC-SEA	Vector Control Consulting – South East Asia
VECAM	Veterinary Entomology in Cambodia

VL	Viral Load
VOC	Variants of Concern
WCS	Wildlife Conservation Society
WEHI	Walter and Eliza Hall Institute of Medical Research
WGBAI	Working Group for Bioinformatics and AI
WGS	Whole Genome Sequencing
WHO	World Health Organization
WHOCC	WHO Collaborating Center on Influenza - Melbourne Australia
WOAH	World Organization for Animal Health
WP	Work Package
WT	Welcome Trust
ZIKV	Zika Virus

8.2 Organizational Chart of IPC



*Including **Community Epidemiology Group, Clinical Research Group, Methodological and Statistical Analysis Group***

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