Surveillance and Data for Management (SDM) Project

Final Report

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ACRONYMS AND ABBREVIATIONS

ACT	Artemisinin-based Combination Therapy
AFP	Acute flaccid paralysis
AL	Fixed combination artemether + lumefantrine
API	Annual Parasite Incidence
ASAQ	Fixed combination artesunate + amodiaquine
CB-CD	Community-Based Continuous Distribution
CFR	Case Fatality Rate
CHM	Central Highlands of Madagascar
CHW	Community Healthcare Workers
CLP	Central Laboratory for Plague
CPN	Consultation Prénatale (prenatal consultation)
CRA	Clinical Research Associate
CSB	Centre de Santé de Base
CSB_R	Centres de Surveillance Biologique Référents
DVSSER	Direction de la Veille Sanitaire, de la Surveillance Epidémiologique et de Riposte
EIR	Entomological Inoculation Rate
EV	Enterovirus
EWS	Early Warning System
fMDA	focal Mass Drug Administration
GIS	Geographical Information System
HBR	Human Biting Rate
HLC	Human Landing Catch
ILI	Influenza Like Illness
IPM	Institut Pasteur de Madagascar
IPTp	Intermittent Preventive Treatment of pregnant women
IRS	Indoor Residual Spraying
ITN	Insecticide-Treated Nets
KMC	Kangaroo Mother Care
LLIN	Long-Lasting Insecticidal Nets
m/p/n	mosquitoes collected by HLC per person per night
MCE	Multi-Criteria Evaluation
MCI	Malaria Control Interventions
MDC	Mass Distribution Campaign
mEWS	Malaria Early Warning System
MOP	Malaria Operational Plan
MoPH	Ministry of Public Health
mRDT	Malaria Rapid Diagnostic Test
MTP	Muirhead-Thomson Pit
MTPC	Muirhead-Thomson Pit Collection

NMCP	National Malaria Control Program
PMI	President's Malaria Initiative
RCD	Reactive Case Detection
RDT	Rapid Diagnostic Test
SARI	Severe Acute Respiratory Infection
SBS	School-Based Malaria Serology Survey
SDM	Surveillance and Data for Management
SI	Sporozoite Index
SSDS	Système de Surveillance Démographique et de Santé
VDPV	Vaccine-derived poliovirus
WHO	World Health Organization

PROJECT OVERVIEW/SUMMARY

Project Name	Surveillance and Data for Management (SDM) Project
Activity Start Date and End Date	October 1st, 2013 to September 30th, 2019
Name of Prime Implementing Partner	Institut Pasteur de Madagascar (IPM)
Cooperative Agreement Number	Grant No. AID-687-G-13-00003
Name of Subcontractors/Sub awardees	None
Major Counterpart Organizations	None
Geographic Coverage (cities and or countries)	Madagascar
Reporting Period	October 1st, 2013 to September 30th, 2019

Introduction

The Surveillance and Data Management (SDM) project (Grant USAID No. AID-687-G-13-00003) was implemented by the Institut Pasteur de Madagascar (IPM) from October 1st, 2013 to September 30th, 2019 in close collaboration with national health authorities and with the support of other USAID partners. This project was designed to support three fundamental purposes: research, training, and health promotion.

The SDM project initially included two work-packages (WPs): WP1 for malaria control, aligned with the President's Malaria Initiative (PMI) and Malaria Operational Plan, and WP2, which focused on mother and child health activities, in line with the health, population, nutrition (HPN) lifecycle of USAID. A third WP was added to support the plague epidemic response of 2017. In total, 32 subprojects (SP) were conducted. They included qualitative and quantitative approaches, encompassing some of the most urgent health issues in Madagascar.

The key results of this project included but were not limited to strengthening the epidemiological surveillance and response system; validation of the current control measures used to fight malaria in Madagascar and the development of new tools to aid the surveillance of malaria outbreaks; determining the etiologies associated with fever, respiratory and diarrhea illnesses in children under the age of 5; identification of certain determinants for the stunting of child growth in Madagascar; and strengthening of the epidemiological surveillance and response to plague.

Consequently, the SDM project has not only successfully contributed to new knowledge but has also raised important questions that will shape the direction of future public health research.

WP1. MALARIA SURVEILLANCE AND CONTROL MEASURES

The objectives of the WP were to strengthen the surveillance systems, support the development of methodologies linking entomological measurements with malaria control programming, and conduct periodic assessments on the effectiveness of malaria control measures, including therapeutic efficacy studies. Operational research using school-based serological surveys were also conducted to validate healthcare center data as a means of prioritizing areas for indoor residual spraying (IRS).

Subproject SP1: Fever sentinel surveillance network

Key leader: Dr Laurence Randrianasolo

Background: In Madagascar, malaria is endemic in approximately 90% of the country and the entire population is considered to be at risk. Malaria is still a public health problem. The National Malaria Control Program (NMCP) changed its policy in 2007 to focus on prevention (with insecticide-treated nets (ITNs) and IRS in epidemic-prone areas), appropriate diagnosis (use of rapid diagnostic tests (RDTs) to identify malaria cases), and treatment of all malaria cases using ACT (artemisinin-based combination therapy). In March 2007, an integrated fever sentinel surveillance system was set up to primarily monitor malaria cases and subsequently those of other diseases. The fever sentinel surveillance system was initially set up in 13 primary healthcare centers ("Centre de Santé de Base" = CSB) in 13 geographically distinct sites. By 2011, the network was comprised of 49 operational sites (34 CSB and 15 hospitals) and by 2018, 54 CSB in 35 districts and 18 district or university hospitals (Figure 1).

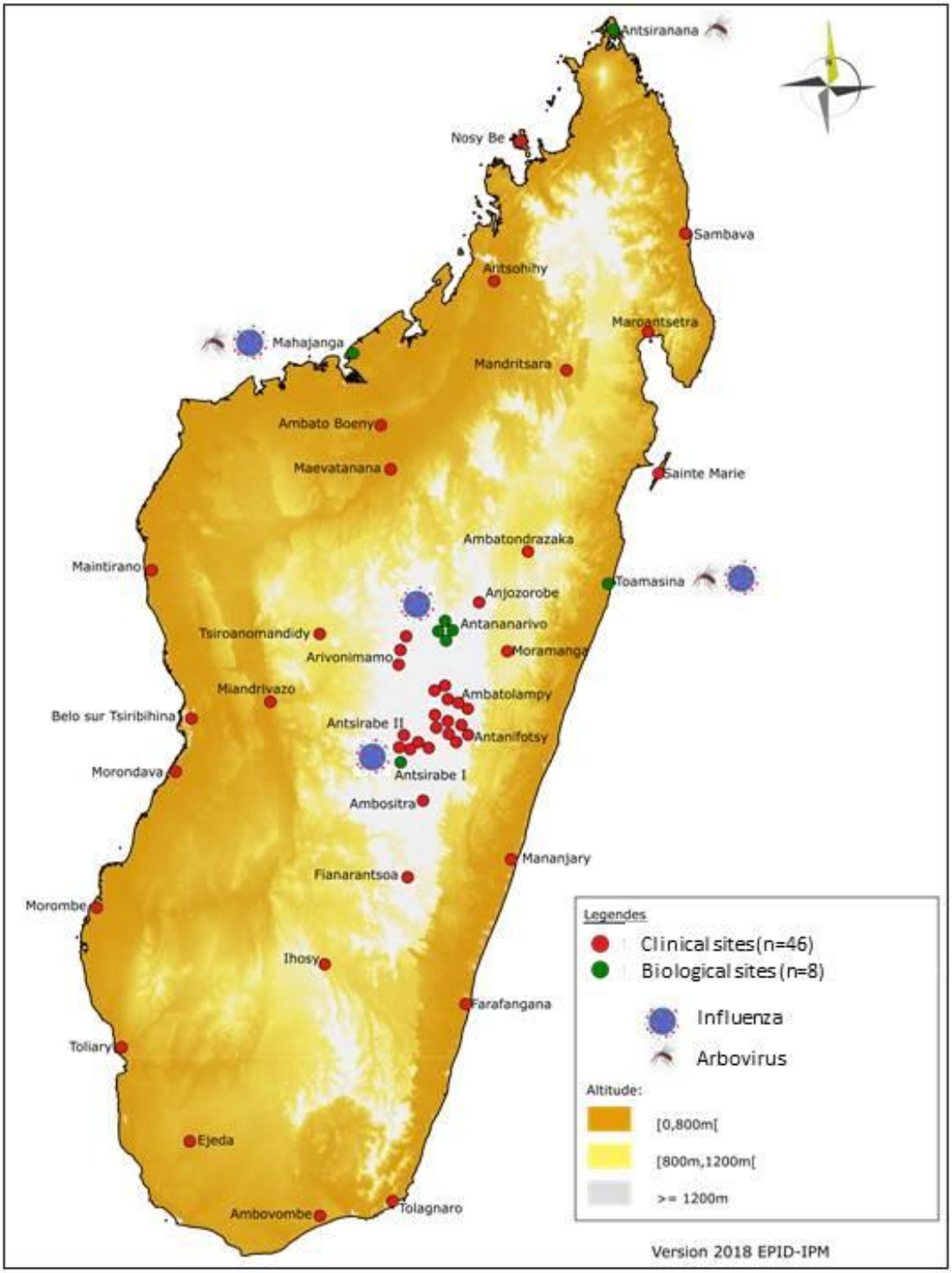


Figure 1. Map of the sentinel surveillance system in Madagascar

In September 2018, the Ministry of Public Health (MoPH) took over management of the entire sentinel surveillance system in the country to harmonize all epidemiological surveillance. The transition of the management was organized with the technical support of the Institut Pasteur de Madagascar (IPM). At that same time, a network of 11 biological sentinel surveillance sites ("Centres de Surveillance Biologique Référents" or CSB_R) was established. All 11 CSB_R currently ensure malaria, polio, plague, measles, food poisoning, and rabies surveillance.

However, among the 11 CSB_R, only seven ensure surveillance for influenza and arbovirus, four ensure surveillance for diarrhea, and only two use microscopy to diagnose malaria cases. The selection of the CSB_R was based on the bioclimate, high population density, lab capacity to treat samples, and area of economic interest and/or local epidemic history.

Study objective: The objective of the sentinel surveillance system is to identify the burden and monitor trends of malaria and other diseases in Madagascar.

Methodology: The fever sentinel surveillance network consisted of a passive syndromic surveillance system coupled with biological surveillance. At consultation, all cases of fever were tested with a mRDT (malaria RDT) and if the result was negative, they were tested using a syndromic approach. Patients were then classified into influenza like illness (ILI), arboviral, or diarrheal syndromes. This information was transmitted daily to the IPM for processing and the production of a weekly surveillance report that was transmitted to the Malagasy health authorities. An android platform was used for data collection and transmission from the CSB_R and a web application for hospital reports. The sentinel sites collected a number of samples per week and sent them to reference centers at the IPM for analysis. The 11 CSB_R currently maintain this biological surveillance activity.

Results: From October 2012 to September 2018, 225 alerts or abnormal situations were detected by the fever sentinel surveillance network: 216 by CSB_R, eight by community healthcare workers (CHW), and one by a hospital. Among the alerts and abnormal situations, 48.0% (108/225) concerned malaria (28 consisted of the identification of autochthonous malaria and 80 of high levels of malaria). In total, 95.1% (214/225) of the alerts and abnormal situations were investigated and controlled by teams from the respective health districts and only 4.9% (11/225) required the support at the central level of the MoPH or multidisciplinary investigation. For malaria outbreaks, the health district team verified the availability of RDTs and ACTs to ensure correct malaria case management and informed the population of the importance of the use of bed-nets.

Impact: Since its inception, data from the sentinel surveillance system have been used to regularly monitor disease trends and have helped to identify several outbreaks in a timely manner, including Rift Valley fever (2008), Chikungunya (2010), A/H1N1pdm influenza (2009), and malaria (2012).

Subproject SP2: RDT quality assurance

Key leader: Dr Laurence Randrianasolo

Background: A fever sentinel surveillance network has been operational since 2007 in Madagascar. To identify malaria cases in all 34 sentinel sites (CSB), all febrile patients were tested with HRP-2/pLDH combination RDTs, according to NMCP guidelines, which can detect all species of *Plasmodium spp*. The RDT performance reported by the manufacturer is based on sensitivity, specificity, positive predictive value, and negative predictive value. These values can change depending on field conditions and malaria prevalence.

Study objective: To assess the performance of malaria RDTs used to survey fever cases through the fever sentinel surveillance network.

Methodology: The evaluation of RDTs was carried out twice a year (during the rainy season and the winter season). Storage conditions (temperature and humidity) and RDT testing, following the manufacturer's instructions, were monitored by a technician or a physician from the IPM. The results of RDTs stored at sentinel sites were compared with those from microscopy and the same RDT batch stored at the IPM at 25°C and below 80% humidity.

Results and discussion: From January 2013 to December 2015, 33/34 fever sentinel surveillance sites throughout the country were visited regularly. There were neither storage errors nor expired RDTs in stock at any sentinel site. Most (81%, 61/75) technicians properly used the RDTs, in accordance with the manufacturer's instructions. The results of 1,635 febrile patients were used for a quality assurance study. Results of on-site RDT use and those stored at the IPM were 99.8% concordant. Comparison with microscopy showed a sensitivity of 92.5%, specificity of 97.1%, positive predictive value of 86.0%, and negative predictive value of 98.5%. These results demonstrate the reliability of the malaria RDT results from the fever sentinel sites. Thus, data collected from the fever sentinel sites can be used by the NMCP to better understand temporal and spatial trends in malaria transmission across Madagascar.

Impact: The elimination of malaria in Madagascar requires an effective epidemiological surveillance system. The quality of data collected by routine surveillance was often limited in terms of completeness and timeliness, but the fever sentinel surveillance network has filled the gap by producing timely data analysis using high quality data. The sentinel surveillance network has provided the opportunity to (i) more accurately monitor malaria trends in Madagascar using differential diagnosis, (ii) identify malaria outbreaks in a timely manner to implement an effective response, and (III) ensure the reliability of diagnostic tools used in Madagascar, including RDTs.

Subproject SP3: Evaluative research of the fever surveillance network system

Key leader: Dr Laurence Randrianasolo

Background: A fever sentinel surveillance system, involving 34 healthcare centers, was set up in Madagascar in 2007 to detect epidemic-prone diseases in real time. Following the World Health Organization (WHO) recommendations for a regular system monitoring, an internal evaluation of the fever sentinel surveillance system was undertaken in 2011 to assess the quality of the generated data and the system attributes. The results showed that the data collected were of good quality.

Study objective: To evaluate the sensitivity of the fever sentinel surveillance system to detect febrile illnesses in Madagascar.

Methodology: The Capture-recapture method was used using two independent data sources: the first source consisted of passive detection of fevers in healthcare centers, whereas the

second consisted of active screening of febrile subjects in the catchment area of each corresponding healthcare center. This method is primarily used to estimate the completeness of notifications and the size of populations in a geographical location. Cases common to both sources were identified by matching name, age, and location. The completeness of collected data was estimated through the population census.

Results and discussion: This study was conducted in 2014-2015 during which time six healthcare centers were randomly selected from the sentinel network. Active screening in the catchment areas of the six healthcare centers detected 2,902 febrile illnesses among 149,835 inhabitants. Acute malaria represented 0.3% (10/2,902) of febrile illnesses. Passive screening in healthcare centers notified 157 cases of fever, of which 7.6% (12/157) were acute malaria. The estimated number of febrile and acute malaria cases in the catchment areas based on the capture-recapture analysis of data from the healthcare facilities and active screening was 3,829 [95% CI: 3,498-4,160] and 17 [95% CI: 7-27], respectively. The overall sensitivity of the sentinel healthcare centers to detect febrile illnesses and acute malaria was 4.1% and 70.0%, respectively. Therefore, most malaria cases were captured by the sentinel fever surveillance system, whereas the fever sentinel surveillance system had a low sensitivity to detect fevers. The proportion of fever cases captured by healthcare centers with medium attendance was threefold higher than that of the other two strata.

Impact: The results will serve as a baseline for future evaluative research of the fever sentinel surveillance system in Madagascar

Subproject SP4: Fever etiology assessment

Key leaders: Dr Julia Guillebaud & Dr Jean-Michel Héraud

Background: Increasing use of mRDTs reveals a growing proportion of patients with fever who do not have malaria. Healthcare workers have few tools to diagnose fevers due to causes other than malaria; a gap between clinical diagnosis, case management, and true fever etiology could lead to partial or even no recovery of the patient. We therefore made a first assessment of the etiologies of fever in Madagascar to ultimately improve the management of febrile cases.

Study objective: To identify the pathogens that may be responsible for fever-causing illnesses in Madagascar.

Methodology: The Fever Sentinel Surveillance Network, in place in Madagascar since 2007, was used to collect accurate and comprehensive clinical data, as well as various specimens from febrile patients. In 2015, 21 sentinel sites located throughout the country, representing all bioclimatic and geographical settings of Madagascar, were selected. Between 30 and 40 consenting febrile patients (with an axillary temperature ≥ 37.5°C according to the Fever Sentinel Surveillance System) were recruited per site. Clinical examinations were performed and several samples taken. Laboratory analyses were selected to reflect a wide range of

infectious diseases that may occur in Madagascar and were performed in collaboration with national and international partners.

Results and discussion: Between April 22, 2014 and September 25, 2015, 685 febrile patients were included in the study, ranging in age from 6 months to 68 years. Children less than 15 years old accounted for 63.2% of total inclusions. We were able to detect at least one pathogen in 42.0% of patients, with 34.9% showing single infections and 7.2% co-infections. Among all patients, 16.9% were confirmed malaria cases and 26.7% had viral infections. Surprisingly, no, or very few, infectious agents largely found in Eastern Africa and Asia, such as zoonotic bacteria or arboviral infection (*i.e.,* dengue), were detected in this study, but an unexpected case of infection with Rift Valley Fever virus was detected. However, we identified pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenza*, and Hepatitis B virus, especially in children, even though they are part of the immunization program. Other co-infections of interest were detected, such as malaria with Epstein-Barr virus (which has been implicated in the development of endemic Burkitt's lymphoma).

These first results raise many questions about the etiologies of community fever in Madagascar. Viral infections represent a significant proportion of causes of febrile illnesses, perhaps because many outpatients are children. Further studies that include concomitant clinical assessment and laboratory investigations, as well as socio-economic behavior in terms of healthcare accessibility should also be implemented. Evaluating the non-systematic use of antibiotics in the management of febrile patients through robust clinical studies should be a priority to avoid not only the risk of adverse events, but also the acceleration of antibiotic resistance.

Impact: This study identified a reasonable proportion of causes of fever in Madagascar. Malaria, respiratory viruses, and other common viruses were, unsurprisingly, the winning trio, with co-infections detected frequently. However, particularities of the country in terms of large bioclimatic variations, diverse ecosystems, and, perhaps most importantly, widely varying accessibility to healthcare, may partially hide a large proportion of infectious agents unknown to be circulating in Madagascar. From a health research perspective, further studies using a more comprehensive clinical, radiological, and laboratory approach are required to generate supplementary indication. From the point of view public health, these findings underline the need for specific diagnostic tools for the effective management of febrile patients and the rational use of antibiotics, as well as the sensitization of both healthcare workers and the population about immunization prevention programs.

Subproject SP5: Mathematical models of surveillance data to detect epidemic thresholds

Key leader: Dr Fanjasoa Rakotomanana

Background: Early detection of outbreaks and rapid control actions are essential to prevent and contain the spread of infectious diseases to reduce morbidity and death. The

implementation of an automated early warning system (EWS) is a key step in adding value to the epidemiological data routinely collected by surveillance systems to improve the timeliness of detection of disease outbreaks.

Study objective: To assess the benefits of a malaria EWS (mEWS), including not only early detection but also forecasting based on a sentinel surveillance system to maximize the potential of the sentinel surveillance system by innovative but simple explorations of population health data, and to provide practical examples and suggestions for use in other systems or settings.

Methodology: Data collected through the 34 fever sentinel sites were sent to the IPM by SMS. The data were stored in a PostgreSQL database on a dedicated server at the IPM. The data included the sentinel site code, date of data collection, total number of outpatient consultations, total number of confirmed malaria cases, total number of ILI cases, total number of dengue-like cases, total number of diarrhea cases, and the number of consultations by age group. Various statistical methods and mathematical algorithms were tested and integrated into the sentinel information system for the early detection of the crossing of epidemic thresholds.

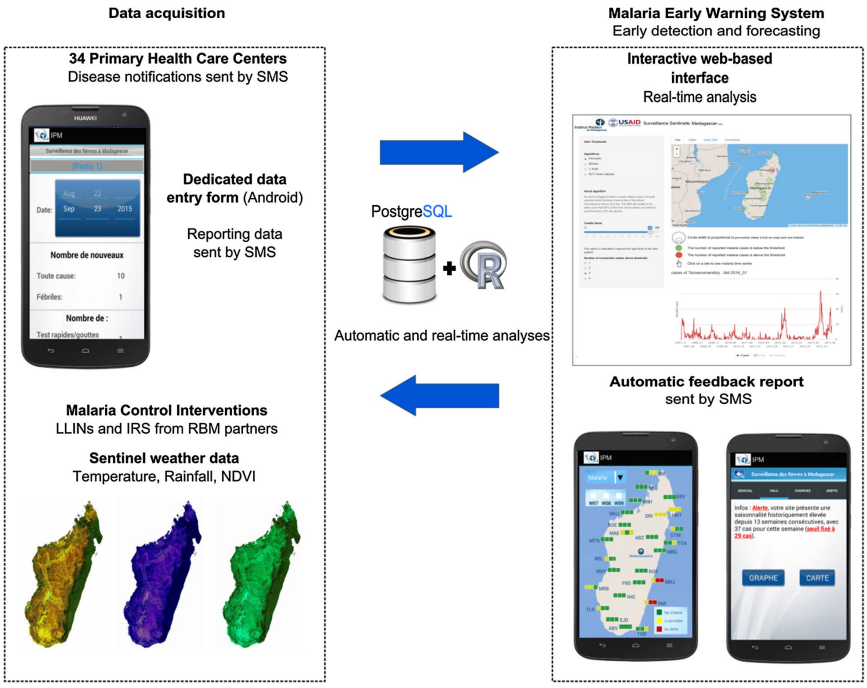


Figure 2. Scheme of the malaria automated early warning system

Results and discussion: A reporting system based on mHealth technology that used Android OS smartphones was developed. The new open-source technology ran through a dedicated application developed by the IPM, involving a handheld data entry device in Malagasy (the national language), a feedback report with automated analysis via charts and maps, and an edutainment-based learning solution. A web-based surveillance system that included EWS and a forecasting model was implemented. The website was accessible to Roll Back Malaria

partners. The development of this automated and graphical mEWS is a promising approach to enhance early outbreak detection and the rapid response capacity in Madagascar.

Impact: The new system allowed the detection of a malaria outbreak on October 6, 2014 in the southeastern region of Madagascar. The web-based surveillance system, with an automated analysis and timely output, allowed real-time monitoring and communication with Roll Back Malaria partners. The mEWS allowed rapid dissemination, interpretation, and subsequent action to control any suspected outbreak. The mEWS system is operational but has not been used (not supplied with data) since the surveillance system was transferred to the DVSSER in late 2018. We are proposing to update the developed plug-in and implement it with the biological surveillance system (CSB_R) depending on the specific surveillance needs of the NMCP (e.g., area in pre-elimination).

Subproject SP6: GIS technology to visualize trends in malaria incidence

Key leaders: Dr Fanjasoa Rakotomanana & Florian Girond

Background: The reduction of global malaria burden over the past 15 years is much attributed to the expansion of mass distribution campaigns (MDCs) of long-lasting insecticidal nets (LLINs). Two LLIN MDCs were held in 2009 and 2012 in Madagascar and the Toamasina district also benefited from community-based continuous distribution (CB-CD) of LLINs. The malaria incidence dropped but eventually rebounded after a decade.

Study objective: To evaluate the effectiveness of mass and continuous LLIN distribution over time in Madagascar.

Methodology: Data from a sentinel surveillance network over the 2009–2015 period were analyzed. Alerts were defined as a weekly number of malaria cases exceeding the 90th percentile value for three consecutive weeks. Statistical analyses assessed the temporal relationship between LLIN MDCs and (i) the number of malaria cases and (ii) malaria alerts detected and (iii) the effect of a combination of MDCs and a CB-CD in the Toamasina District.

Results and discussion: The percentage of alert-free sentinel sites was 98.2% during the first year after LLIN MDC, 56.7% during the second year, and 31.5% during the third year. Analyses showed an increase of 13.6 and 21.4 points in the percentile value of weekly malaria cases during the second and the third year following the MDC of LLINs, respectively. The number of weekly malaria cases decreased by 14% during the CB-CD in the Toamasina District. In contrast, sites without continuous distribution showed a 12% increase in malaria cases.

The study results support the effectiveness of LLIN MDCs in malaria prevention in Madagascar. Indeed, MDCs were almost all followed by a drop-in the number of malaria alerts across the sentinel surveillance system. However, the duration of such protection appears to be limited to one malaria season if not reinforced with continuous LLIN distribution (Girond Florian et al., 2018). Mass campaigns are a cost-effective way to rapidly achieve high and equitable coverage, but experience shows that coverage gaps emerge almost immediately post-

campaign due to deterioration of the nets, the loss of nets, and population growth. Several studies have shown that net decay is highly variable and that the average 'lifespan' may be considerably less than three years. Our results suggest that these declines in coverage and effectiveness translate into a reduced impact on the number of malaria cases. Given the various issues affecting coverage gaps, health policymakers should redirect their attention to both LLIN quality and distribution plans, optimizing coverage to maximize impact on morbidity and most likely mortality.

The analysis from the sentinel site of Toamasina suggests that a combination of MDCs followed by community-based continuous distribution of LLINs can succeed in maintaining a low number of malaria cases for several years.

Impact: The sentinel surveillance system in Madagascar provided real-time evaluation of the impact of malaria control interventions at both national and regional levels. Information on the duration of the impact of LLINs at the population level will further help malaria control programs to define the optimal timing of subsequent net distribution campaigns. As e-health technologies develop, surveillance data is increasingly available and similar analyses could be conducted at a lower cost in other countries. In 2012, a similar sentinel surveillance system was implemented by the Institut Pasteur Dakar; this system is mainly focused on Influenza-like illness (Ndongo Dia et al, 2014). A scale-up of e-health solutions, as currently occurring in Madagascar, opens the way to more solid designs, such as cluster randomized trials (CRTs). The use of routine sentinel surveillance to monitor and assess the effectiveness of malaria control interventions over time could easily guide malaria elimination strategies.

Subproject SP7 GIS and vector control program to identify priority areas for indoor residual spraying

Key leader: Dr Fanjasoa Rakotomanana

Background: Many types of interventions have already been carried out to control malaria in Madagascar. In areas of high malaria prevalence, control measures for malaria include ITN, IRS, MIP, and case management at community and healthcare facilities, as implemented by the Malagasy NMCP. Malaria vector control and prevention in Madagascar is based on the use of LLINs and IRS. Before 2013, IRS was the main form of vector control in the Central Highland Regions. LLINs were distributed mainly in coastal regions. The 2016 MIS showed that LLIN universal coverage was 44% and 54% in the districts of endemic areas. Malaria risk modeling and mEWS are used to assess the risk of malaria in Madagascar, particularly in epidemic-prone areas. In low-income countries, such as Madagascar, the selection of areas targeted for IRS is based on the availability of logistics and the assessment of a risk gradient for the population and the environment.

Study objective: To identify priority regions for IRS based on risk-gradient determination. Specifically, this study was conducted to map malaria risk in the central highland regions of Madagascar and to validate the results by comparison with epidemiological data.

Methodology: GIS (geographical information system) and multicriteria evaluation (MCE) were used for this study. MCE is a common method for assessing and aggregating many criteria. To form a single index of evaluation and provide necessary information for decision makers, MCE basically consists of combining information from several criteria: population density, distance to wetlands, temperature, elevation, precipitation, and inhabited zone. It is considered to be a semi-quantitative method using a participatory approach in which stakeholders bring their knowledge and expertise. Outputs from modeling were compared to data on annual parasite incidence (API) provided by the NMCP. These data record the incidence rate of malaria per commune and per year.

Results and discussion: Three malaria risk models were tested in 2014, 2015, and 2016. Given the lack of API validation data, only the malaria risk model of 2016 was validated. Validation showed an acceptable fitting, with an AUC of 0.736 (95% confidence intervals (CI): [0.669–0.803]) (Rakotoarison Anthonio et al., 2020). A "full MCE" plugin tool was developed that is free and user-friendly (Figure 1). This plugin groups the main steps for MCE evaluation: reclassification of constraints, normalization, computing the weight for each factor using a pairwise comparison matrix, and aggregation of factors. Staff members at the PNLP and DVSSER, researchers from the IPM, and University of Antananarivo students were trained to use this tool in 2017 and 2019. The tool was also transferred to the NMCP for use.

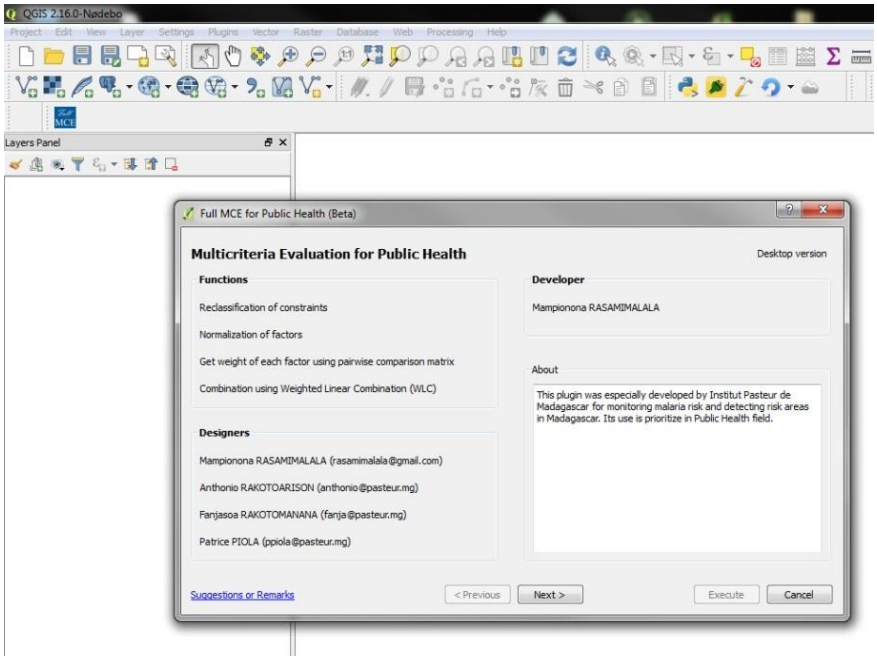


Figure 3. Interface of the developed multicriteria evaluation For Public Health (MCE) Plug-in

Impact: GIS combined with MCE, through their capacity for storage, data management, analysis, modeling, and mapping of spatially referenced data, is a useful tool to capture geographical decision issues. The resulting risk map is used for decision-making to target priority communes on which to focus IRS campaigns in Madagascar. The "Full MCE for Public Health" tool, which is dynamic, rapid, and easy to use, should be easily appropriated by

decision-makers to prioritize IRS and its flexibility makes it easy to use in other contexts, for other diseases, and other countries to simulate various scenarios.

Subproject SP8: Anopheline mosquito monitoring in the districts of Farafangana, Miandrivazo, and Morondava, Madagascar, 2014-2017: evaluation of the malaria transmission risk and implications for vector control

Key leaders: Dr Thiery Nepomichene & Dr Romain Girod (former key leaders: Dr Fara Raharimalala & Dr Sébastien Boyer)

Background: Malaria vector control and prevention in Madagascar is based on the use of LLINs and IRS. In Madagascar, LLIN mass campaigns started in 2008, whereas IRS was implemented in the late 1940s. Before 2013, LLINs were distributed mainly in the coastal regions, whereas IRS was carried out in the Central Highlands. This strategy was based on the knowledge of malaria transmission patterns. Indeed, in the coastal regions, malaria is endemic and stable, with a long period of transmission (sometimes persistent throughout the year), whereas in the Central Highlands and margins, as well as in the southern regions, malaria is classified as unstable, with shorter periods of transmission and a greater risk of epidemic emergence. However, variations in malaria infection have been observed in certain regions, attributable to the efficient use of LLINs and IRS. Extension of both LLINs and IRS was carried out and a combination of the two methods was implemented in certain parts of Madagascar. Now, IRS is carried out in certain parts of the coastal region, as well as LLINs.

Anopheles arabiensis, *An. funestus*, *An. Gambiae*, and *An. mascarensis* are the main malaria vectors in Madagascar. Other species, such as *An. coustani, An. merus*, and *An. Squamosus*, are often abundant in the human environment and found to be associated with malaria parasites in natural conditions.

Entomological indicators, such as the species composition of the *Anopheles* population, vector densities, biting rates of vectors, their biting and resting behaviors (biting times and proportion of vectors resting or biting indoors and outdoors), the parity rate, and the longevity of female populations, as well as their infection rates, have been missing or have required an update in many areas of Madagascar. Moreover, these indicators may change over seasons and years following the massive use of LLINs and IRS, as observed in other countries, whereas precise knowledge of these indicators is essential to define the best strategic orientations for malaria vector control.

Study objective: To provide a picture of the mosquito composition and the density of the malaria vector populations over the seasons and years; to describe their dynamic behavior from numerous key entomological indicators, including human biting rates (HBRs) and parity rates; and to evaluate their role in malaria transmission through the evaluation of their natural infection rates.

Methodology: Entomological surveys were carried out every two months between 2014 and 2017 in three districts showing different patterns of malaria transmission: Farafangana,

Miandrivazo, and Morondava. Mosquito collection was performed using human landing catches (HLC) and Muirhead Thomson pit collection (MTPC). Mosquitoes were identified in the field and the parity rate estimated. Infection of the mosquitoes with *Plasmodium spp* was determined using an ELISA test for *Plasmodium* Circum Sporozoite Protein (CSP). Malaria entomological indicators, such as HBR, parity rates, sporozoïte indices, and entomological inoculation rates were estimated.

Results and discussion: This longitudinal survey allowed the assessment of certain entomological parameters in the districts of Farafangana and Morondava. Limited information was obtained from the Miandrivazo district, as the sampling was conducted during just one year, in 2014. Specifically, the anopheline fauna composition, malaria vector behavior, and malaria transmission risk were characterized, allowing the orientation of vector control measures.

Concerning the anopheline fauna, we identified 14 *Anopheles* species in the Farafangana and Miandrivazo districts, indicating high specifies richness, whereas only eight *Anopheles* species were identified in the Morondava district. In the Farafangana and Miandrivazo districts, four known major malaria vector species were collected: *An. funestus, An. gambiae, An. Arabiensis*, and *An. mascarensis*. In addition, two other anopheline species were found to be abundant: *An. coustani* and *An. squamosus.* In the Morondava district, two known major malaria vector species, *An. funestus* and *An. arabiensis,* and three other anopheline species, *An. merus, An. Coustani*, and *An. squamosus* were collected.

The density of the main malaria vectors fluctuated throughout the year. Some vectors were abundant at the beginning of the rainy season and others during or at the end of the rainy season. *Anopheles arabiensis* and *An. gambiae* were generally abundant at the beginning of the rainy season (in January), whereas *An. funestus* was abundant during and at the end of the rainy season. Exceptionally, *An. gambiae* was found to be abundant during the dry season in September in the Farafangana district.

All anopheline species showed a tendency towards exophagic behavior, except *An. funestus* in the Farafangana district. Indeed, outdoor HBRs were significantly higher than those indoors, mainly for *An. coustani*. In addition, a significant proportion of biting activity was observed both early in the evening, before 10:00 pm, and late in the morning, after 3:00 am for this species. During these periods, inhabitants are often outside for various reasons. These behaviors could help malaria vectors elude vector control methods. Indeed, in the Farafangana district, where IRS was conducted each year, usually in September and October, no notable diminution of the outdoor HBR was observed. However, the parity rate decreased in September relative to that in July and November. An increase in the parity rate was measurable from January. For *An. funestus*, which is mainly an endophagous species, we observed diminution of the indoor HBR, associated with a diminution of the parity rate, during September and November in the Farafangana district. No effect of vector control could be estimated in the Miandrivazo district, as the sampling was conducted over only one year. However, in the Morondava district, the density of anopheline species drastically decreased from September 2015 throughout 2016 and even 2017 following a mass campaign distribution

of LLINs that was carried out in the fourth quarter of 2015. By contrast, there was no effect on the vector parity rate. Indeed, in the Morondava district, the parity rate was high compared to that in the Miandrivazo and Farafangana districts. Moreover, for *An. arabiensis* and *An. coustani,* the parity rate indoors was slightly higher than that out outdoors and the parity rate in 2016 was significantly higher than that in 2015. Therefore, the density of the vector population decreased but the risk of malaria transmission remained at high levels, as the population was old. Finally, *An. arabiensis* and *An. coustani* were found to be proven vectors of malaria in the Morondava district. In the Farafangana district, *An. funestus*, *An. gambiae*, *An. Mascarensis*, and *An. coustani* were found to be responsible for the malaria transmission.

Impact: The findings obtained during this study show that malaria vectors are present in the three districts and the risk of malaria transmission remains high, even after vector control measures were implemented. Indeed, the efficacy of vector control is diminished by malaria vectors that adopt an exophagic behavior and biting at atypical hours. Thus, supplementary methods to those currently applied must be identified and tested urgently in for use in the field to significantly decrease malaria transmission.

Subproject SP9: Insecticide resistance testing, approaches, and methodologies developed to link entomological measurements and malaria control

Key leader: Sanjiarizaha Randriamaherijaona & Dr Sébastien Boyer

Project terminated

Subproject SP10: Anopheline mosquito monitoring in the Central Highlands of Madagascar, 2014-2017: evaluation of the malaria transmission risk and implications for vector control

Key leader: Dr Thiery Nepomichene & Dr Romain Girod (former key leaders: Dr Fara Raharimalala & Dr Sébastien Boyer)

Background: In the highlands of Central and Eastern Africa, malaria is less prevalent, sometimes absent, above 1,500 m of altitude, where climactic features vary. The major difference in transmission intensity is associated with altitude because of the associated changes in temperature, which affect the development of both the parasites and the vectors.

In Madagascar, malaria remains a major public health problem despite policy efforts to control the disease. The country possesses various epidemiological facies, ranging from high-level perennial transmission in the coastal regions to seasonal transmission, with the risk of epidemics in the central highlands and southern part of the territory. This complex situation is due to the diversity of biotopes, resulting in a multiplicity of vector species and their bio-ecological characteristics.

In the Central Highlands of Madagascar (CHM) and its fringes, malaria is unstable, limited by temperature and the presence of a sloping topography (limiting the development of breeding sites), with high inter-annual variation, depending on the temperature and precipitation. The CHM are generally situated at an altitude above 1,200 m and experience alternating wet and hot seasons (from October to April) and dry and cold seasons (from May to September).

At the beginning of 1980, an increase of malaria cases diagnosed in the dispensaries of the CHM was noted. Between 1985 and 1990, several malaria cases occurred in the suburban region of Antananarivo, suggesting endemic transmission. Since 1999, blanket IRS has been replaced by targeted IRS in restricted areas. During the last decades, interventions were limited because of the scarcity of autochthonous transmission episodes. *An. funestus* is presently considered to be the major vector of *Plasmodium falciparum* malaria in the CHM and *An. arabiensis* a secondary vector, but recently *An. coustani* was also found to be a vector.

Entomological surveys have been conducted in areas with different patterns of malaria endemicity and different ecological profiles, allowing the evaluation of the impact of control strategies. Nevertheless, entomological indicators, such as species composition of the *Anopheles* population, vector densities, biting rates, biting and resting behaviors (biting times and proportions of vectors resting or biting indoor and outdoor), parity rate, and the longevity of female populations, as well as their *Plasmodium* infection rates, are lacking or need to be updated in many areas of Madagascar. Moreover, these indicators may change over seasons and years following the massive use of LLINs and IRS, as observed in other countries, whereas precise knowledge of these indicators is essential to define the best strategic orientations for malaria vector control.

Study objective: 1) To understand the spatial distribution and composition of the mosquito fauna and the density of the malaria vector populations during the seasons and years; 2) to describe their dynamic behavior through a number of key entomological indicators, including human biting rates and parous rates; and 3) to evaluate their role in malaria transmission through the above-mentioned indicators and the evaluation of their natural infection rates.

Methodology: Entomological surveys (mosquito collections) were conducted in 11 villages belonging to five districts of the CHM at three timepoints during one or two malaria transmission seasons from 2014 to 2017. Both HLC and MTPC were used and the mosquito collection effort was strictly similar at each collection session in each village.

Results and discussion: Anopheline mosquitoes were collected in all 11 villages, including all known major malaria vector species described in Madagascar, except *An. Merus*, which is usually present in coastal areas, namely *An. arabiensis, An. gambiae, An. Funestus*, and *An. mascarensis.* Several known secondary malaria vector species, such as *An. coustani, An. rufipes, An. Flavicosta*, and *An. pharoensis*, were also collected by HLC, as well as accidental malaria vectors, such as *An. squamosus, An. maculipalpis,* and *An. pretoriensis*. Anopheline species diversity was variable depending on the villages, from only three species collected in Ambatomiady to 14 species collected in Andramy.

Densities were also variable depending on the village, the month, and species, ranging from 25 mosquito bites per person per night (m/p/n) for *An. arabiensis* in December 2016 to more than 40 m/p/n for *An. coustani* in April 2017 in Tsimadilo. *An. arabiensis* was the predominant known major malaria vector species in most villages, with highest abundance at the beginning of the rainy season in December or February, and *An. coustani* was the most predominant known secondary malaria vector species, with the highest abundance at the end of the rainy season, in April. *An. gambiae* was predominant at the beginning of the rainy season, whereas *An. funestus* and *An. mascarensis* were predominant at the end of the rainy season, in April, and until June or July, depending on the village.

All these predominant *Anopheles* species exhibited mainly exophagic behavior, except *An. funestus,* which was found to be more endophagous during certain months in Andramy and Androtra.

Biting cycles also varied depending on the village, the month, and species. Some species, including several known major malaria vectors, exhibited biting activity before 9 pm or after 3 am. In particular, *An. coustani* exhibited an evident peak of activity before 9 pm in most villages, as well as a trend to bite early in the morning.

Variation in parity was observed across villages, months, and species. A trend of progressive increase in the parity rates during the transmission season was observed in most species, especially *An. arabiensis*, which showed the highest parity rates in June of July in most villages. There were, however, several exceptions, for example for *An. coustani* and *An. arabiensis*, which showed high parity rates in February in Ankily and Andramy, respectively.

Naturally *Plasmodium*-infected anopheline mosquitoes were collected in 7 of the 11 villages. All four major malaria vectors known to be present in the CHM were found to be positive, except *An. funestus*. The predominant *An. arabiensis* was found to be positive in four villages, with a sporozoite index (SI) from approximately 0.10% to 0.20% and an estimated entomological inoculation rate (EIR) from approximately 4.0 to 8.0 infected bites per human per year. Infected *An. arabiensis* were mostly collected at the beginning of the rainy season, in December or February, both indoors and outdoors, during the middle of the night. The two other known major malaria vectors, *An. gambiae* and *An. mascarensis*, were found to be positive only in the village of Andramy, with a SI of 1.1% and 3.7%, leading to estimated EIRs of 4.1 and 8.4 infected bites per human per year, respectively. Infected *An. gambiae* and *An. mascarensis* were collected outdoors at 10 to 11pm in April and June. The incriminated malaria vector *An. coustani* was found to be positive for *P. falciparum* sporozoites in four villages with a SI from approximately 0.3% to 1.7%, leading to an estimated EIR of 4.0 infected bites per human per year in Ambohidrangory and 56.0 infected bites per human per year in Tsimadilo. Infected *An. coustani* was collected mostly in April, predominantly outdoors, with approximately 50% of bites occurring early in the evening or late in the morning. Two other anopheline species, known as accidental malaria vectors, were found to be positive for *P. falciparum*. *Anopheles squamosus* was found to be infected in three villages, with a SI from approximately 0.2% to 1.5%, leading to an estimated EIR of approximately 4.0 infected bites per human per year. This species was collected throughout the malaria transmission season

both indoors and outdoors at various times throughout the night. *An. maculipalpis* was found to be infected in the only village of Androtra, with a remarkable SI of 3.7%, leading to an estimated EIR of 4.0 infected bites per human per year. The only infected specimen was collected outdoors between 10 and 11pm in July.

Collections in MTP consisted primarily of *An. Arabiensis*, which was collected mostly in December or February, suggesting exophilic behavior of this species at the beginning of the season.

These findings suggest that vector control must be at its optimum efficacy from the early beginning to the late end of the rainy season in the CHM. The known major malaria vector *An. arabiensis* appears to be the primary vector at the beginning of the season. This species bites both outdoors and indoors throughout the night and could be the target of current vector control tools, i.e., IRS and LLIN, even if it shows a trend towards exophagic and exophilic behavior.

Impact: The study showed that the incriminated malaria vector *An. coustani* appears to play a potential role in malaria transmission in the CHM, especially in April. Its atypical behavior, biting early in the evening and in the morning, mostly outdoors, suggests that the current vector control strategy is not adapted to this species, which could be responsible for residual malaria in the CHM. In such a situation, in addition to IRS and LLINs, it is recommended to develop and implement complementary vector control tools that target exophagic and exophilic vector species.

Subproject SP11: Quarterly malaria bulletin to summarize key findings and indicators from the sentinel system

Key leader: Dr Laurence Randrianasolo

A quarterly malaria bulletin was produced by the IPM and RBM partners to ensure the sharing of information on the epidemiological situation of malaria in Madagascar.

The bulletin integrated malaria data collected by the various existing surveillance systems: fever sentinel surveillance, specific surveillance for malaria, and integrated surveillance of diseases operated by the MoPH.

The 3 to 4 pages of the malaria bulletin illustrated and interpreted data. It included summary tables, maps, graphs, and curves resulting from the malaria surveillance systems.

In June 2017, the NMCP began to produce the malaria monthly bulletin in collaboration with USAID Measure Evaluation phase IV project. The IPM continues to support the editorial team.

Subproject SP12: Epidemiological study and monitoring/evaluation to improve malaria control interventions and strategies

Key leader: Dr Thomas Kesteman

Background: Madagascar, as do other malaria endemic countries, depends mainly on international funding for the implementation of malaria control interventions (MCI). As these funds are no longer increasing, policy makers need to know whether these MCI actually provide the expected protection.

Study objective: This study aimed to measure the effectiveness of MCI deployed in all transmission patterns of Madagascar in 2012 to 2013 against the occurrence of clinical malaria cases.

Methodology: From September 2012 to August 2013, patients consulting for non-complicated malaria in 31 sentinel healthcare centers (SHCs) were asked to answer a short questionnaire about LLIN use, IRS in the household, and intermittent preventive treatment of pregnant women (IPTp) intake. Controls consisted of healthy individuals of all ages sampled from a concurrent cross-sectional survey conducted in areas surrounding the SHC. Cases and controls were retained in the database if they were residents of the same communes. The association between *Plasmodium* infection and exposure to MCI was calculated by multivariate multilevel models and the protective effectiveness (PE) of an intervention was defined as 1 minus the odds ratio of this association.

Results and discussion: Data from 841 cases (out of 6760 cases observed in the SHCs) and 8,284 controls were collected. Most samples were from the Western transmission pattern (61.4% of cases, 52.2% of controls), then the East (30.0% of cases and 26.5% of controls), and Fringe (6.9% of cases and 8.1% of controls); the Highlands and the Southern transmission patterns encompassed seven and eight cases only (representing 0.8 and 1.0% of cases, respectively) and their controls represented 8.1 and 5.1% of controls, respectively. In the areas in which LLINs are distributed, i.e., the Eastern, Western, Southern, and Fringe transmission patterns, the sample encompassed 31 clusters, including 834 cases and 7,617 controls. The use of LLINs every night was higher for controls (53.2%) than cases (39.3%) and provided a significant 53% PE (95% CI [20–73]) in bivariate and 50% (95 % CI [16–70]) in multivariate analyses. IRS campaigns took place in the Fringe, most of the Highlands, and certain parts of the Western and Southern transmission patterns. In these areas, living in a household that had been sprayed within the last 12 months provided a significant 54% PE in bivariate and multivariate analyses (95% CI [30–70] and [36–68], respectively). In clusters in which IRS campaigns and LLIN distribution occurred, being exposed to both MCIs provided a significant 72% PE in bivariate and multivariate analyses (95% CI [22–90] and [28–89], respectively. In areas in which IPTp was proposed, having taken at least one dose of IPTp during pregnancy provided 78% PE in bivariate analysis and 73% PE in multivariate analysis, but these results were not statistically significant. Deployment of LLINs and IRS may have prevented 100,000 cases annually (Kesteman et al; 2016).

Impact: This case–control study provided reliable information for guidance in policy making. In Madagascar in 2012, both LLINs and IRS provided good protective effectiveness against clinical malaria. These results may apply to other countries with similar transmission profiles, but such case-control surveys could also be recommended to identify local failures in the effectiveness of MCI.

Subproject SP13: Use of malaria serology to validate healthcare facility-based data for prioritizing IRS in the Central Highlands of Madagascar

Key leader: Dr Inès Vigan-Womas

Background: Malaria remains endemic in 90% of Madagascar, although the entire population is considered to be at risk for malaria. Much progress has been made in reducing the malaria burden in Madagascar in the last decade. However, transmission has increased recently in certain areas, and focal outbreaks have increased over the last two years. IRS with effective insecticides is one of the primary approaches of vector control to reduce the malaria burden and prevent malaria outbreaks in epidemic-prone areas.

The Central Highlands and Fringes areas of Madagascar experience low malaria transmission, which is estimated to be approximately 1% according to a household survey that tested children aged less than five years (MIS report 2013). The Central Highlands and Fringes areas have a long history with IRS for malaria control funded by the PMI and the Global Fund to Fight AIDS, Tuberculosis, and Malaria. After four years of blanket spraying (of all communes in the selected districts), the NMCP changed its policy to focalized spraying, targeting the communes in selected districts with the highest malaria incidence based on healthcare facility data. Concerns about the completeness and accuracy of healthcare facility data are compounded by low rates of care seeking in the formal sector in Madagascar, raising questions about the validity of the current approach to estimating transmission intensity and prioritizing communes for IRS. Serological markers of malaria exposure may be one of the most suitable methods of detecting malaria hotspots in low-transmission settings. Antibodies to malaria can persist over time and can represent a more stable measure of malaria transmission than parasite prevalence, which can vary substantially by season.

Study objective: To identify the simplest and most cost-effective methods for characterizing transmission intensity in Madagascar for the purpose of IRS stratification. School-based malaria serology surveys (SBS) were used as a gold standard to validate the recently implemented approach of using healthcare facility-based and routinely reported malaria incidence data to prioritize communes for focalized IRS, in addition to evaluating the performance of several additional potential measures of transmission intensity.

Methodology: To achieve this goal, a SBS was conducted in the 107 communes of the seven districts localized in the Central Highlands and Fringe zone of Madagascar, which have had four consecutive years of annual IRS, plus one year of focalized spraying supported by the PMI. Serological markers of malaria exposure from children and their parents from two schools in

each commune served as gold standard measures of malaria transmission intensity for the commune against which healthcare facility data and absenteeism data were compared.

Results and discussion: RDT positivity was very low, at 0.53%, ranging from 0% (66/93 (71%) communes) to 13.3% by commune. The global seroprevalence by commune for *Plasmodium falciparum* antigen PfMSP1 was 18.2% (range: 3.0% to 63.0%) and that for PfAMA1 26.5% (range: 4.4% to 70.4%). The correlation between seroprevalence and malaria incidence at the commune level was 0.46 for MSP1 and 0.45 for AMA1, $p < 0.001$ for each.

Healthcare facility data identified 21 of 30 communes with the highest transmission according to MSP1 and AMA1 (sensitivity = 70% (95%CI: 50-85%)).

Impact: Healthcare facility surveillance data was relatively reliable for identifying areas of higher transmission but missed 30% of malaria foci. In low malaria endemic settings, sero-epidemiological studies using *Plasmodium* biomarkers represent sensitive tools to monitor the intensity malaria transmission and guide malaria control programs.

Subproject SP14: Monitoring the therapeutic efficacy of artesunate and amodiaquine for treating uncomplicated malaria in Madagascar

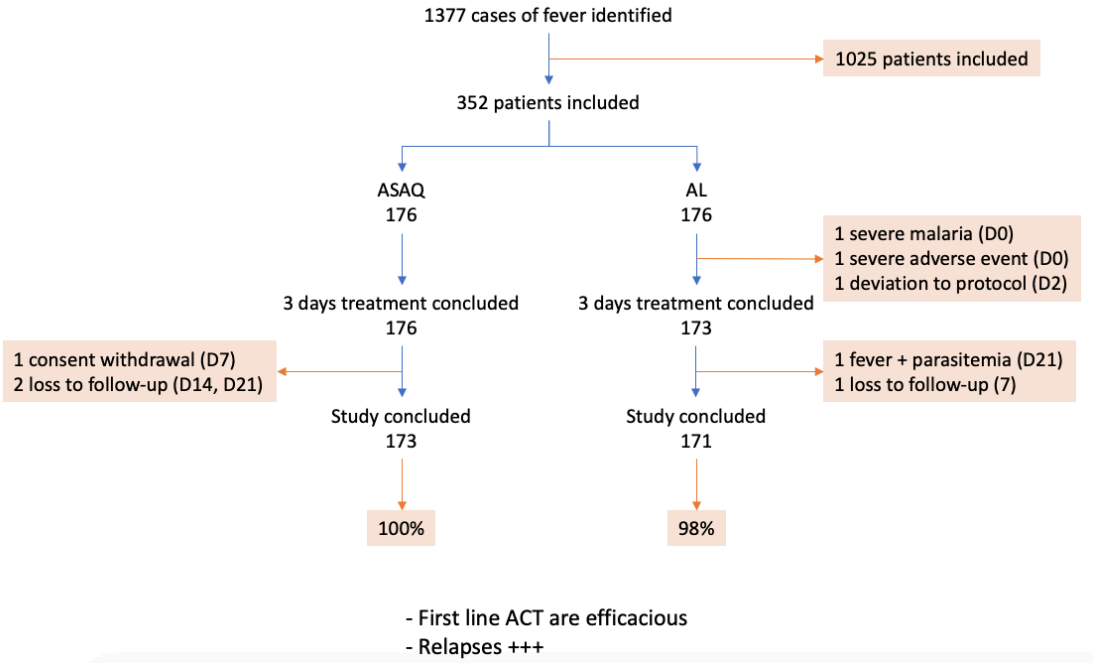
Key leaders: Dr Judickaelle Irinantenaina & Pr Milijaona Randrianarivelojosia

Background: In Madagascar, antimalarial drugs (ACT) play a key role in achieving the elimination of malaria. The efficacy of ASAQ (fixed combination artesunate + amodiaquine) for treating *P. falciparum* uncomplicated malaria has been known since 2006. ASAQ is commonly used at healthcare facilities and is also available at the community level for malaria case management. The emergence of artemisinin resistance in *P. falciparum* malaria in South East Asia represents a considerable threat to global health. Thus, it is crucial to monitor ACT therapeutic efficacy in Madagascar.

Study objective: To generate relevant information on ASAQ and AL (fixed combination artemether + lumefantrine) efficacy for treating *P. falciparum* malaria in Madagascar.

Methodology: The NMCP and the IPM were involved in this project. They separately conducted the study using the same protocol at two sites each (NMCP managed sites in Marovoay and Vangaindrano and IPM managed sites in Mananjary and Farafangana). Data were collected from April to September 2018. There were two study arms at each site: (i) Arm 1: treatment with ASAQ and (ii) Arm 2: treatment with AL. Consenting parents of children 6 months to 15 years of age and assenting patients (> 7 years of age) suffering from uncomplicated *P. falciparum* malaria (parasite load from 1,000 to 100,000/µl of blood) were randomly assigned to a treatment arm. After a 28-day follow-up, the parasitological (crude and PCR-corrected) and clinical responses were assessed.

Results and discussion:



Impact: The study demonstrated the efficacy of ASAQ and AL as treatment strategies in Madagascar.

Subproject SP18: A comparison of reactive case detection and focal mass drug administration strategies to provide information to the national malaria control strategy towards elimination

Key leader: Dr Aina Harimanana

Background: Malaria is endemic in Madagascar. The CHM, situated at 1,000 meters of altitude, experienced an outbreak of malaria with high mortality in the '80s. In 2016, the prevalence of Plasmodial infection among children aged between 6 and 59 months was 0.9%, indicating low transmission of malaria. Nevertheless, this area is still prone to malaria epidemics due to the presence of mosquitos and breeding sites in the CHM.

The WHO recommends community-based interventions to reduce malaria transmission, including various forms of active case detection, which can include mass screening and treatment in higher transmission settings, as well as more focalized screening and treatment in low and very low transmission areas. The WHO also recommends that surveillance systems in very low transmission and pre-elimination settings conduct reactive case detection around passively detected index cases. Active case detection can help find and treat community members with clinical malaria earlier than they would otherwise have sought care, as well as those with asymptomatic malaria infections who would otherwise remain infectious. Since malaria can be geographically localized, active case detection in targeted areas can be cost-effective as transmission declines. One highly focalized form of active case detection is reactive case detection, screening and treating community members in a given radius around

a passively detected index case, which can be efficient for finding and treating additional cases. The updated national malaria strategy calls for (re)active case detection in the pre-elimination districts, which was one of the arms of the study.

Results from recent studies have found that screen-and-treat strategies have not reduced malaria transmission as much as anticipated, primarily due to the limits of detection of RDTs. Interest in (re)active case detection and mass drug administration has increased recently as a result of a renewed focus on elimination malaria, but some questions remain about their effectiveness and their relative acceptability and feasibility in low-transmission settings. Therefore, to enable the NMCP to refine future strategies, one arm of the study will assess the impact of focal mass drug administration, which does not depend on parasite detection by RDTs and therefore is more likely to eliminate sub-microscopic infections.

Study objective: To compare malaria annual parasite incidence (API) in the CHM over a period of two years in a reactive case detection (RCD) arm and a focal mass drug administration (fMDA) arm compared to a control arm.

Methodology: The study was conducted in three different phases:

1. Census survey: collection of the study population to ensure the quality of measuring the AIP (main indicator of the study) by establishing the denominator of the calculation. This survey lasted two months in the 39 clusters.

2. Cross-sectional study: this part of the study consisted of an evaluation of plasmodial prevalence by PCR examination. The target was to estimate the variation of the parasite pool in a population sample and evaluate a proportion of plasmodial infections not detected by mRDT but detected by PCR. One hundred individuals per cluster were randomly selected from census data. The study was conducted over three months. The cross-sectional study was planned to be carried out in three phases (Year 0, Year 1, and Year 2), but only the baseline has been performed.

3. Enhanced survey: the survey included three arms with 13 clusters per arm:

    - RCD arm: screening of the malaria cases by mRDT of all household members within a radius of 100 m around the index case (malaria case detected in a focal healthcare center) and treatment of all mRDT positive cases.

    - fMDA arm: treatment of all household members within a radius of 100m around the index case (not tested).

    - Control arm: detection of malaria cases in the focal healthcare center (no survey in the household).

Results and discussion: For the initial population survey, at least 5,000 individuals from approximately 15 km around a healthcare center were identified for each cluster (the cluster radius can be higher or lower than 15 km, depending on the population density). During this

phase, the team surveyed all households around the center until reaching the objective. An average of 5,299 individuals per cluster was recruited. Overall, 206,669 individuals were identified.

For the cross-sectional study, survey teams were able to reach 3,792 individuals (97%) of the targeted population. Overall, 2,952 (76%) accepted to take part in the survey. The PCR analysis detected 20 cases of malaria infection. Among the 20 cases detected by PCR, five had a negative mRDT.

Due to a lack of funding, the enhanced survey was conducted for only three months. During this period, 1,210 patients came to the healthcare centers for fever (control arm: 369, RCD: 402, fMDA: 439). Among them, 77 had a positive mRDT (index cases): 22 in the control arm, 30 in the RCD arm and 25 in fMDA arm. The index cases came from 24 clusters in 14 districts.

Impact: The first results show that, even during the winter season, autochthonous malaria transmission is present in the CHM. Thus, RCD or fMDA could be part of a key strategy to achieve malaria elimination in these areas. Further investigations need to be performed to confirm the appropriate strategy. The complexity of setting up such a trial in a low-income country should not be underestimated and would require a careful feasibility assessment with a budget adapted to the field conditions to collect high-quality data.

Subproject SP21: Bio-efficacy monitoring of long-lasting insecticidal nets in Madagascar

Key leader: Dr Thiery Nepomichene & Dr Romain Girod

Background: Malaria prevention with LLINs has seen a tremendous scale-up in sub-Saharan Africa in recent years. As many countries have now achieved high coverage of their population with LLINs and are approaching the WHO-recommended universal coverage target of one net for every two people of the population at risk, the question of how such high coverage levels can be maintained has been raised. In this context, the WHO guideline is to monitor "net durability" to determine the frequency at which nets need to be replaced and the type of net to be procured and prioritize adequate replacement operations.

Madagascar is composed of 22 regions with more than 25 million inhabitants and a surface of 587,000 km². The geography of the island results in a tropical climate, characterized by rainy and dry seasons of different lengths, depending on the region. Most rural areas are not accessible by car, as the roads are in poor condition. These situations explain the heterogeneity of the epidemiology of malaria and affect the logistics of the distribution of mosquito nets.

Net durability monitoring is based on three indicators: 1) net survival, which is the percentage of nets still present and in use in the household to which they were distributed, 2) physical integrity, quantification of the size and number of holes in the LLIN, and 3) bio-efficacy, a measure of the insecticidal effect of LLINs on mosquitoes, especially anopheline mosquitoes responsible for malaria transmission. As stated by the WHO guidelines, LLINs should have

adequate insecticidal activity after 20 standard washes and a minimum of three years under routine use in the field.

Study objective: To evaluate the bio-efficacy of the LLINs distributed in Madagascar and sampled by PSI Madagascar.

Methodology: A mass distribution campaign of LLINs took place in September 2015 in Madagascar. A net durability study was conducted in four districts that received PermaNet® 2.0 nets. Bioassays were performed on PermaNet® 2.0 nets upon their arrival in the territory, at 3 to 6 (baseline), 12 and 24 months post-distribution. Additional bioassays were performed on DawaPlus® 2.0 and Yorkool® nets at 24 months post-distribution. All bio-efficacy studies were carried out with the susceptible *Anopheles arabiensis* strain reared in the IPM insectary to evaluate the intrinsic efficacy of the nets, as recommended by the WHO.

Additionally, bio-efficacy analysis was performed on DawaPlus® 2.0 and PermaNet® 2.0 LLINs distributed in September 2018. Bioassays were performed upon arrival in Madagascar and one month after distribution (baseline). All bio-efficacy studies were carried out with the susceptible *An. arabiensis* strain reared in the IPM insectary. Additionally, samples of certain tested nets were also sent to the CDC to assess the insecticide residues on the nets by chemical analysis.

Results of the bio-efficacy studies are intended to be complementary to the study of survival and physical integrity conducted by the IPM.

Results and discussion: The bio-efficacy of new PermaNet® 2.0 nets upon their arrival to the territory in 2015 was acceptable according to the WHO criteria concerning anopheline mosquito mortality rates. Moreover, monitoring conducted on PermaNet® 2.0 nets showed that in-the-field bio-efficacy after 3 to 6 months of use was still acceptable, but that they lost their bio-efficacy after 12 months and *a fortiori* 24 months of use. Yorkool® and DawaPlus® 2.0 nets distributed during the 2015 mass campaign were tested only 24 months after use in the field. These nets were not bio-effective according to the WHO criteria concerning anopheline mosquito mortality rates.

The bio-efficacy of new DawaPlus® 2.0 and new PermaNet® 2.0 nets upon their arrival to the territory in 2018 and after one month of use in the field was acceptable according to the WHO criteria concerning anopheline mosquito mortality rates. Moreover, bioassays carried out on new DawaPlus® 2.0 nets and after one month of use in the field showed that they did not meet the WHO criteria in terms of knock-down effect. In addition, chemical tests that assessed the insecticide residues on the new bioassayed DawaPlus® 2.0 nets distributed in 2018 and the DawaPlus 2.0 nets collected after one month of use in the field showed that the quantity of deltamethrin on the nets was below the expected values (mean quantity of deltamethrin < 80 mg/m²).

The results show that the tested mosquito nets distributed in 2015 no longer had the required biological efficacy at the 12th month of use in the field. This may be due either to the quality of the insecticide impregnation in the factory, the conditions of transport or storage of the

mosquito nets, or the specific conditions of use of these mosquito nets by the Malagasy population.

The evaluation of the DawaPlus® 2.0 nets distributed in 2018 should continue and other types of LLINs should be tested.

Impact: These results are of great interest for the malaria vector control program. Indeed, they show that the main strategy of the distribution of PermaNet® 2.0 nets by mass campaigns every three years is problematic in Madagascar. Continuous distribution of nets in Madagascar may remedy this problem. In addition, it may be informative to conduct in-depth studies to understand why the bio-efficacy of mosquito nets used in Madagascar is shorter than that declared by the manufacturers.

Subproject SP30: The role of *Anopheles coustani* in malaria transmission in Madagascar

Key leader: Dr Thiery Nepomichene & Dr Romain Girod

Background: Malaria remains endemic in Madagascar. The transmission of the disease is heterogeneous throughout the Island. Four species of anopheline mosquitoes (*Anopheles gambiae, An. funestus, An. Arabiensis*, and *An. mascarensis*) are known to be involved in transmission. However, other species, such as *An. Coustani*, are widely distributed in many parts of the country and may be locally very abundant. Recent studies showed this species to be infected with human *Plasmodium* parasites and it is suspected to play a role in the local transmission of malaria, although this is yet to be confirmed. In addition, preliminary data relative to this species showed that its biting behavior (times and places of bites) is variable and locally atypical, suggesting that *An. coustani* may partially evade classical vector control measures deployed in Madagascar (use of LLINs and IRS). Further studies should be conducted accordingly.

Study objective: To highlight the natural infection of *An. coustani* in the field and its ability to transmit malaria parasites in the lab.

Methodology: Mosquitoes were collected in two districts of Madagascar, Farafangana and Maevatanana, using HLC and CDC simple light traps (LT). Mosquitoes resting indoors were also captured by spraying non-remnant insecticide. Mosquitoes were identified morphologically in the field and upon arrival to the laboratory and PCR and ELISA were used to identify the species of mosquito and Plasmodium infection. The HBR was determined to evaluate the exophagic/endophagic behavior of the various anopheline species, as well as their aggressiveness cycle. Natural infection of anopheline mosquitoes by Plasmodium species was also investigated to measure the sporozoite index (SI) for each species. The vector competence of *An. coustani* was also assessed in the laboratory by artificially infecting females with the blood of malaria patients carrying gametocytes.

Results and discussion: *An. coustani* was among the more abundant species and was found to be positive for *Plasmodium falciparum* in the districts of Maevatanana and Farafangana.

The SI observed for this species in the village of Amboangisay (district of Farafangana) showed that it may contribute to the transmission of malaria. Moreover, as observed previously in other areas of Madagascar and other Sub-Saharan countries, *An. coustani* appears to be an exophilic and exophagic species. No specimens of this species were found resting indoors during the day and its biting activity was observed mainly outdoors. In addition, the species bites humans mainly early in the evening and early in the morning. In the village of Amboangisay (district of Farafangana), all 13 specimens found to be positive for malaria parasites were collected outdoors, of which 62% were collected before 10:00 pm and 23% after 3:00 am.

Impact: The behavior of *Anopheles coustani* behavior may allow this species to evade the classical vector control measures based on LLINs and IRS, which are designed for endophilic and endophagic species that are primarily active at night. In this context, it is advisable to design complementary methods of vector control and prevention that could overcome these difficulties.

Although no infected and infective *An. coustani* females were obtained (no oocysts) during the experimental infections (six infections in total), we recommend that the assessment of vector competence of *An. coustani* in the laboratory continues, especially because certain results were inconclusive.

WP2. INCREASED EFFECTIVENESS OF NUTRITION AND MATERNAL, NEWBORN, AND CHILD HEALTH INTERVENTIONS (MCH)

In Madagascar, as in many other developing countries, malnutrition is one of the main problems affecting infancy and early childhood. The objectives of the WP were to carry out nutritional research, analyze the behavioral determinants of stunting, and design and issue recommendations for interventions to address it.

Subproject SP15: Qualitative research to identify the primary two or three sociocultural/behavioral determinants of stunting and develop strategies to address these determinants and recommendations for implementation in USAID project sites

Key leader: Dr Chiarella Mattern

Background: Madagascar has the fourth highest rate of stunting in the world, with a disparity between regions. Over the past few years, stunting has gained international attention because of its severe and irreversible short- and long-term consequences, which include poor cognition and school performance, low adult wages and productivity, and an increased risk of nutrition-related chronic diseases and mortality.

Study objective: This socio-anthropological survey was undertaken to identify the sociocultural determinants of stunting in Madagascar.

Methodology: This study was conducted from November 2014 to March 2015 in three districts of Madagascar: Moramanga, Farafangana, and Morondava.

Semi-structured interviews were carried out with mothers and grandmothers of children showing both stunted and normal growth aged between 0 and 24 months, along with traditional birth attendants, midwives, community health/nutrition workers, and traditional healers. Focus groups were conducted with fathers.

Results and discussion: In total, 153 semi-structured interviews, 15 focus groups with fathers, and 63 direct observations of child feeding practices and household hygiene were conducted. The results of this study suggest that a multitude of local perceptions and practices influence childcare and feeding practices within households. More specifically, our results show that beyond the problems related to food insecurity, there is a direct link between local perceptions and the nutritional status of children. For example, local beliefs about colostrum, breast milk, and rice are deeply rooted in the population's beliefs, especially in rural areas. This could explain the high stunting rates in areas such as Moramanga where there is high food availability.

Stunting remains an unrecognized form of malnutrition among most of the general population of Madagascar and among certain healthcare professionals. Most participants explained the "small height" of their children as a genetic legacy and a consequence of physical activity. It is

therefore considered to be a normal condition. At this point, being underweight and wasting are the only well-recognized forms of under nutrition. Grandmothers and traditional birth attendants (TBA) play a significant role in the healthcare and nutritional practices of mothers and infants, in particular, in the spreading of messages. We noticed that mothers are highly dependent on the influence of grandmothers concerning these particular issues. Fathers have little or no influence on childcare and feeding practices within households. Most of the mothers who stated that the grandmother represented the main source of information related to childcare and nutrition instead of a healthcare professional had a stunted child. In addition, most infants aged from 6 to 24 months who were fed complementary foods before or at four months of age were stunted. Finally, most of the stunted children were recurrently ill with diarrhea and fever.

Impact: The present study was performed to determine the sociocultural determinants of stunting. The results provide a number of answers related to the ancestral and sociocultural practices surrounding pregnancy, breastfeeding, and complementary feeding practices. Our results show the role of elders in the circulation of traditional messages and food practices (i.e., grandmothers or traditional birth attendants) and how these messages are passed on from generation to generation. Based on our findings, we propose operational recommendations that can be used to improve nutritional programming and strategies in the fight against stunting in Madagascar.

Subproject SP16: Research on malnutrition and opportunistic infections to develop therapeutic guidelines on malnutrition complicated by opportunistic infection(s)

Key leader: Dr Rindra Randremanana

Background: In Madagascar, chronic malnutrition is the main problem of malnutrition; for almost 25 years, the proportion of children under five years of age with stunted growth has remained at almost 50%. Moreover, even though the country is in a phase of epidemiological transition, infectious diseases are still the main cause of under-five mortality. In 2016, according to the WHO, the three main causes of death in children under five years of age in Madagascar were pneumonia (21%), malaria (20%), and diarrheal diseases (17%), excluding neonatal cases. The two-way relationship between malnutrition and infection is well known. Thus, the prevention or control of malnutrition should take into account that of infectious diseases and vice versa.

Study objective: The purpose of this study was to propose management recommendations for chronically malnourished children with infection and to identify the determinants of feeding practices and chronic malnutrition.

Methodology: Determinants of chronic malnutrition complicated by parasitic intestinal infections were identified among children aged 6 to 59 months in two districts: Moramanga at the level of the SSDS (Système de Surveillance Démographique et de Santé) of the population and in Morondava within 13 Fokontany of the Commune of Bemanonga. Data

were collected from January to July 2014 in Moramanga and from August to November 2014 in Morondava.

Intestinal infections of parasitic origin are common opportunistic infections of chronically malnourished children.

Malnourished children less than five years of age were initially screened using measurements of weight, height, and brachial circumference. Malnourished and normally nourished children aged 6 to 59 months were then randomly selected from those who participated in the screening for inclusion in a case-control study. Cases were represented by chronically malnourished children and controls were normally nourished children. A questionnaire was administered to mothers or usual caregivers of the children.

To collect data on potential determinants of chronic malnutrition, stool samples were collected to test for intestinal parasites.

Results and discussion: Among 9,330 children who had valid anthropometric measurements in the two districts, 1,826 aged between 6 and 59 months were included in the case-control study by drawing lots: 894 children in Moramanga (431 chronically malnourished children and 463 children without malnutrition problems) and 932 (420 chronically malnourished children and 512 children without malnutrition problems) in Morondava. At the Morondava site, the proportion of children with inadequate feeding practices (index obtained from breastfeeding, dietary diversity, and frequency) was higher than in Moramanga and concerned half of the children aged 6 to 35 months. On the other hand, more than three quarters (77.5%) of children from Moramanga showed inadequate energy intake. This situation could be explained by the higher consumption of staple foods (cereals, tubers, and roots), as well as legumes; these two food groups contributing 72.0% and 7.0%, respectively, to the daily energy intake of Morondava children. In all cases, the diets of the children at the two study sites showed deficiency for lipids, proteins, and micronutrients. The higher prevalence of inadequate feeding practices among the children recruited in Morondava was related to the higher frequency of mothers with no education (39% vs 11% in Moramanga), as well as the low use of the healthcare system during prenatal visits; 28% of the mothers did not make an antenatal visit to a healthcare worker in Morondava, whereas this proportion was less than 10% in Moramanga. Our results highlight the role of the mothers' positive practices (prenatal consultation, more time spent with children), their level of education, and their experience in determining the level of children's feeding practices. The role of women's education in improving the quality of children's nutrition was confirmed in this study.

The prevalence of chronic malnutrition was higher than the national average (47%) in Moramanga, 52.8% (95% CI: 51.7-54.0). In Morondava, the proportion of chronic malnutrition was 40.0% (95% CI: 37.8-42.1). The results of stool examinations for intestinal parasites showed the presence of at least one investigated parasite in 23.6% of the enrolled children. The prevalence of parasitic infections was 28.4% in Moramanga and 18.3% in Morondava. The most commonly identified parasite in Moramanga was *Ascaris lumbricoides* (16.1%), followed by *Trichuris Trichiura* (3.8%). In Morondava, *Entamoeba coli* (6%) and *Hymenolepis nana* (4%)

were the most prevalent parasites. Infections with more than one parasite (mixed infection) were found in 11% of infected children (2.5% of all studied children), 13.4% in Moramanga and 7.6% in Morondava. In Moramanga, we found evidence that the child's age, Trichuris trichiura infection, and the household wealth index were predictors of child stunting. Data from Morondava, suggested that higher age of the child, the mother's working status, and low birth weight were risk factors for stunting, whereas increased birth spacing was a protective factor. As the determinants of chronic malnutrition reported in our study confirmed its multifactorial nature, the strengthening of interventions should involve different sectors. Programs with solid healthcare access, safety nets, hygiene, and sanitation components should efficiently reduce stunting in Madagascar. However, a strong political commitment matched with multi-sectoral collaborations and wider program coverage are necessary.

Impact: In conclusion, our analysis confirms that stunting remains a major public health problem in Moramanga and Morondava. After many years of neglect, stunting is now considered to be a major global health priority. In Madagascar, the new nutrition plan (Plan National d'Action III 2017-2021-PNAN III) aims to reduce the prevalence of chronic malnutrition from 47% to 38% by 2021. All the stunting determinants identified in this study are targeted by the current PNAN III and are key components of the Sustainable Development Goal: e.g., to eradicate poverty (Goal 1), to ensure good health and well-being (Goal 3), to empower women and girls (Goal 5), and to ensure availability of clean water and sanitation (Goal 6).

Subproject SP17: Research and/or field-testing on new cost-effective innovations or potential interventions that support improvements in maternal, newborn, and child survival to clandestine abortions

Key leader: Dr Rila Ratovoson

Background: In Madagascar, Article 317 of the Malagasy penal code and Article 28 of Malagasy Law 2017-043 condemn women who induce abortions and all individuals (medical or not) who help them. Studies have shown, however, that abortion is frequently practiced in Madagascar. The rate of abortion is 20.4% in the capital and 10.6% in rural areas across all ages among women who become pregnant between the ages of 15 and 49. In 2010, complications of abortion (16%) were the second leading cause of maternal deaths in healthcare facilities in Madagascar after ante- and postpartum hemorrhage (19%). In 2015, they were reported to be the fourth leading cause of maternal deaths (11.8%), after sepsis (23.6%), hemorrhages (19.4%), and uterine rupture (15.3%) in the health sector development program (PDSS 2015).

Most studies in Madagascar have been based on surveys of women hospitalized for complications or health records. However, not all women who have complications resulting from illegal abortions seek medical help for various reasons. Amongst them, are fear of being reported to the judicial authorities or financial reasons. It is currently difficult to precisely determine the proportion of abortions performed by matrons (traditional birth attendants) or

the mothers themselves. To know the exact burden of disease due to induced abortions, community studies must complement studies conducted in hospitals and healthcare facilities.

This study included a quantitative and qualitative component.

Quantitative study

Study objectives: To assess the prevalence of abortion complications at the community level for women aged 18 to 49 living in rural and urban areas of Madagascar. The methods used and actors associated with abortion were determined, and the risk factors for abortion complications were studied.

Methodology: This study was conducted at the Gyneco-Obstetric University Hospital Center of Befelatanana and the maternity Center of the Moramanga Level II District Hospital Center. Patients admitted for post-abortion care were invited to participate in the study from May 4th to August 15th, 2015.

A community-based study was also conducted among women of reproductive age to collect and analyze information on abortion through interviews in 10 districts in Madagascar from September 2015 to April 2016. These individuals were distributed throughout various regions in the north: Sambava, Vohemar, south: Tulear, Ambovombe, east: Toamasina, Maroantsetra, west: Mahajanga, Mitsinjo, and center: Antananarivo and Moramanga. To handle the refusals and underreporting that may occur during home interviews, a second component consisted of submitting the same questionnaire to women of childbearing age attending family planning consultations in public or private healthcare facilities or a consultation with the matrons (traditional birth attendants) residing in the same study area as the community-based study (10 districts).

Results and discussion: In the hospital setting, 308 patients seeking post-abortion care were included. Among them, 104 reported having had an abortion, 25 had an infection (26%), and four died due to abortion. The factors associated with the risk of developing an upper genital infection after induced abortion were the performance of at least one end uterine maneuver to induce the abortion (OR = 12.81 [2.21-73.99]) and the use of a public center as the first facility (OR = 11.86 [1.74-80.90]). The use of a medical method (OR = 0.09 [0.01-0.66]) protected the women from upper genital infection. Factors associated with life-threatening complications were the abortion itself (OR = 0.34 [0.14-0.80]) and the use of a medical method (OR = 0.36 [0.15-0.87]). Performing a diagnostic or therapeutic procedure before admission (OR = 3.62 [1.03-12.7]) also presented a life-threatening risk.

For the community-based study, 19,510 individuals were registered, of whom 3,466 (17.7%) were women of childbearing age. Nearly 91% (3,180 women) agreed to participate in the study, with an average age of 30.7 years (standard deviation 8.9). Among them, 3,063 were sexually active, with the youngest being nine years of age at first intercourse and one-quarter (25%) by the age of 15. Among these 3,063 women, 1,013 (33%) reported having had at least one spontaneous or induced abortion during their fertile life and 11.5% (352/3,063) reported

having had at least one induced abortion in the last 10 years before the investigation. The results of the study showed that young women (18-24 years old) with an education level of middle or high school, living in urban areas, who accept sex for gifts or money were the most at risk of having an induced abortion. Women who had induced abortions were more likely to have used contraceptive methods. Complications of abortion were more frequently reported by women who had spontaneous abortions and they sought care more often than those who reported having induced abortions. Concerning the complications of induced abortions, those who accepted gifts in return for sexual intercourse were the most likely to have had complications. The place of residence (urban/rural) was not related to the occurrence of induced abortion complications.

In healthcare facilities (public or private) in the surveyed areas, women who consulted for family planning were contacted to participate in the study. There were only 317 women who accepted to participate in the 10 districts. Among them, data from 309 women were analyzed. The average age at first intercourse was 17 years, with a minimum of 11 years. Among the 309 women, 59 (19.1%) reported having had an abortion in the last 10 years, of which 38 (12.3%) were induced. Sixty-six episodes of abortion were recorded, of which 43 were induced. The average number of induced abortions per woman was 1.1. On average, the age of the fetus at the time of induced abortion was 2.2 months, ranging from 1 to 6 months maximum. Concerning the methods used, the results on the use of invasive methods were similar to those in the community survey. The insertion of urinary catheters or tubes is still practiced by medical personnel.

The results of the study showed that invasive procedures (vaginal tube insertion, uterus curettage and cleaning) were more frequently performed by medical staff, whereas these methods had the highest risk of complications. The lack of skills of abortion providers, the lack of respect for aseptic rules, and the clandestine nature of carrying out abortions may be determinants that favor abortion complications as long as induced abortions remain strictly prohibited and, indeed, it remains a public health problem in Madagascar. Young women are most at risk and complications are not considered to be a serious and urgent problem requiring immediate care.

Impact: Despite the illegal nature of induced or clandestine abortion, it is an omnipresent practice in Madagascar. An abortion, whether spontaneous or provoked, can often lead to serious complications. Young women are most at risk of induced abortions. The combination of surveys in the medical and community settings made it possible to assess the prevalence and factors related to the occurrence of abortion complications in our study, such as youth and financial insecurity. Special attention should be given to women serving as domestic workers, as they are not only generally young but, above all, of low socio-economic status. In parallel, young female students should also benefit from better sex education to minimize the potential for risky behavior.

Qualitative study

Study objectives: To identify the risk factors for abortion complications by studying women's socio-economic and family background, reproductive history, and the relationship between their abortion complications and contraceptive methods, as well as the logic underlying their decisions when seeking - within or outside the healthcare system - either an abortion or healthcare after the appearance of the first signs of complications.

Methodology: An anthropological study was conducted in 2015 and 2016 in two distinct areas of Madagascar (Antananarivo and Tulear) comprising both rural and urban settings. In depth semi-structured interviews were conducted with 60 women who had had an abortion, 20 healthcare providers, and 20 traditional practitioners.

Results and discussion: This study highlights the diversity of abortion practices and methods in Madagascar and the diversity of actors involved in the practice of abortion. Women can abort alone, but they almost always seek advice from a relative or do so with her/his help (e.g., spouse, friend, mother, or sister). In other cases, women abort with the help of one or more healthcare actors, be it private or even public healthcare professionals (e.g., midwives, doctors, nurses) or traditional healers.

Although there are various abortion methods available, women generally do not have a choice. However, there is still a method available for which they have easier access. For example, if the woman lives in Antananarivo, it may be possible for her to get access to misoprostol. If she knows a *Reninjaza*, known for "getting the menses back," she can go see her. If she knows traditional methods passed down from generation to generation of women (such as vaginal insertion of a plant stem), she can implement this type of method. Women's misunderstanding of the expected effects of "normal" abortion also increases their risk of complication, as is their lack of knowledge of how to use certain methods, such as misoprostol. Our study highlights the multiple modalities by which misoprostol is used for abortion in terms of the dosage (number of tablets, number of doses, and spacing), the mode of administration (oral or vaginal), and especially the terms of pregnancy, even when misoprostol is prescribed in a biomedical setting by a healthcare professional. These results suggest a lack of information on the use of misoprostol for abortion for women and healthcare professionals.

Nevertheless, misoprostol appears to play an important role in abortion practices, as one-third of the women surveyed used it. Its accessibility (in pharmacies, with or without a prescription, drug depots, and from sellers in the informal market), low cost, perception of ease of use, independent of the intervention of a third party and therefore conferring autonomy, and perception of high efficiency all explain the frequent use of this method of abortion in both urban and rural areas.

Most post-abortion complications occurred when the surveyed women used the services of healthcare professionals (except for certain women in rural areas who practiced self-medication or who went to a traditional healthcare practitioner). However, the time interval between the first symptoms and the medical consultation was sometimes long (several

women had bleeding for several weeks before consulting a doctor). This delay in care is due to several causes: ignorance of the women, the shame of having an abortion, or the fear of a bad reception or negative reaction by the caregiver. In addition to these findings, the study highlights the complicated relationship between women and the management of reproductive life: few women were satisfied with the contraceptive method used (side effects noted, regular method changes, low confidence).

Impact: In terms of recommendations, it is necessary to better inform women about the risks of abortion, the functioning of their bodies, and the cycles of reproduction; to train professionals in the management of post-abortion complications and the advice to be given to patients suffering from complications (particularly in terms of contraception); strengthen family planning, especially for young women, and increase awareness among men; increase access to emergency contraception; and empower women and their right to decide about their own reproductive lives.

Subproject SP19: Qualitative study in malaria: ownership and use of long-lasting insecticide-treated bed nets in Madagascar

Key leader: Dr Chiarella Mattern

Background: Although a decline in the mortality rate due to malaria has been observed over the last decade, malaria is still a major public health problem in Madagascar. The distribution of LLINs through universal coverage campaigns is a widely adopted approach for large-scale malaria prevention.

Study objective: To identify factors within households that affect LLIN ownership and use.

Methodology: In total, 64 in-depth interviews and 64 direct household observations on the use of LLIN/bed nets were conducted in LLIN recipient households 6 to 10 months following the 2015 LLIN mass campaign distribution in 2016. Interviews were carried out with household heads (men) and women from various family configurations. The criteria of eligibility were pregnancy (primigravida women), age of the children (under 5 years of age and between 10 - 18 years) and having had children who already left the house. These criteria were chosen to survey a wide range of practices concerning the nets according to the stage of life. Participants were recruited through a two-step process whereby all houses in the community were listed first and then randomly selected for the recruitment of eligible household members. In addition, the "photovoice" process and focus groups were used to identify images associated by participants with malaria and LLINs.

Results and discussion: The results of our survey show that interviewed individuals had a good knowledge about malaria and a relatively positive image of LLINs. However, there was a lack of knowledge on transmission. The use of bed nets/LLINs for all members all year long was first conditioned by the ownership. Survey participants mentioned problems that occurred during the distribution, leading to a lack of distributed LLINs. Heat was mentioned in all areas as a barrier for use (from October to December). The use of bed nets was affected by the

perception of vulnerability of certain family members (particularly pregnant/nursing mothers and children under 5) and the organization of sleeping spaces and associated taboos. The seasonal calendar of economic activities, social events, and cultural factors also determine LLIN too. The ack of appreciation of the type of net received also led populations to not use them. Indeed, the study showed that the color, shape, and fabric of bed nets determined their use. Inadequate mosquito net maintenance was also observed. In all areas, many survey participant washed their LLINs with local powder soap, such as “Klin", and/or dried them under the sun. As a consequence, the protective effects of the bed nets may have been compromised within only a few months after their distribution.

Old nets were used until they were perceived to be ineffective in the protection against insects. The newly received LLINs were not directly used. They were first stored (for an indefinite period ranging from 3 to 12 months) until those which were actually in use were considered to be ineffective.

Our results indicate that such beliefs and practices limit the use of newly received LLINs, in addition to inadequate maintenance practices of the mosquito nets. Such factors may contribute to a reduction in the positive impact of mosquito nets on public health.

Although the results of our interviews revealed a good general knowledge of malaria and LLINs, obstacles remain in the use of LLINs. Therefore, efforts should be made to enhance the knowledge of community workers and the population about the link between mosquitoes and malaria, the importance and effectiveness of LLINs to protect against malaria, and the necessity of using the new nets soon after distribution.

Moreover, our study shows that men and young people feel generally less affected by the disease. Therefore, men and single young men should be more involved and particularly targeted by sensitization campaigns on the importance of using bed nets.

In response to problems in the distribution of mosquito nets, we suggest a more in-depth analysis with institutional and field actors who are involved in the distribution of LLINs from the central to household level to understand the points at which mosquito nets are lost.

Impact: Our study provides the basis for selecting the nets to be distributed (color, shape, mesh size), considering people's preferences and perceptions of insecticide-treated nets to ensure greater use (target distribution: September 2017). We recommend strengthening the continuous distribution of mosquito nets. The non-use of nets for protection against malaria also depends on the preferences of the population in terms of the quality of the nets. Given the importance of the seasonality of certain economic practices (vanilla harvest, fishing) and the link between these practices and the non-use of LLINs, we recommend taking into account the calendar of such practices for the distribution of mosquito nets.

Subproject SP20: A cluster randomized trial to assess the efficacy of fortnightly malaria RDT testing at the community level in high-transmission areas

Key leader: Dr Rila Ratovoson

Background: From December 2016 to January 2018, the IPM implemented PECADOM Plus in 22 rural fokontany or hamlets (administrative limits of a village) in the District of Mananjary in the south-east region of the country. This area is considered to be endemic for malaria transmission. The study was conducted in close collaboration with the Case Management Service of the NMCP from the MoPH.

Study objective: To measure the effectiveness of malaria case management in homes to reduce the prevalence of malaria cases by comparing the data collected during the initial and final household survey. Additionally, this project was also designed to measure the prevalence of anemia among women of childbearing age (15 to 49 years).

Methodology: This study was conducted in the district of Farafangana. Among the 22 rural fokontany chosen, 11 were randomly selected as the intervention arm and the remaining 11 as the non-intervention (control) arm. During the baseline survey (December 2016 to February 2017), trained interviewers, in collaboration with CHWs and CRAs (Clinical Research Associates), conducted the baseline survey among all participating residents from both arms. Trained interviewers were field agents recruited specifically during the baseline and endline surveys to strengthen the CHWs. They were not necessarily nurses or healthcare workers but investigators with a bachelor's degree level education. CRAs were nurses or midwives recruited for the entire study period whose role was to supervise the CHWs and verify and manage detected serious cases requiring transfer to the closest healthcare centers. The baseline survey included: census of the population and GPS registration of all participating households, socio-demographic questionnaires to all household residents, screening by RDT for malaria prevalence of all residents (with and without fever) who agreed to participate in the study, as well as testing for anemia of all participating women of childbearing age. If a resident was diagnosed by a positive RDT, treatment was administered according to the current NMCP drug recommendation, ACT. If a woman was diagnosed with anemia, she received care according to the national recommendations on anemia from the MoPH of Madagascar.

Throughout the period of the intervention (March to October 2017), the CHWs conducted bi-weekly screenings in the intervention arm to identify any fever cases or notions of fever within the last two days prior to the visit. When an individual reported fever, the temperature was taken and an RDT was performed. If the RDT was positive, the individual was treated with ACT at no cost. Patients with negative RDTs or a fever > 39.5˚C and/or other signs of severity, children under two months of age, and pregnant women, regardless of the RDT results were referred immediately to the closest healthcare center. All data during this period was directly collected by the CHWs. These activities were performed under close supervision by the team of CRAs and data managers and researchers based at the IPM.

During the endline survey (November 2017 to January 2018), trained interviewers, CHWs, and CRAs conducted socio-demographic questionnaires for all household residents and screening by RDT was again performed for both arms, as well as anemia testing for women of childbearing age. If either the RDT was positive or anemia was diagnosed, the individual received treatment at no cost.

Results and discussion: The proactive community-based case management of malaria cases by CHWs for all age groups has already shown its efficacy in programmatic approaches, such as in Senegal and Mali. This study demonstrated tis efficacy through a randomized cluster trial in a malaria endemic rural area of Madagascar.

Among the study population, 48% were children below 15 years of age, of whom 53% were female. The educational level was quite low, with 91% of the population not attending middle school. After the 2015 LLIN campaign, 89% of the participants reported sleeping under an LLIN. IRS was used in four and six fokontany in the intervention and non-intervention arms, respectively.

After the randomization of 11 fokontany per arm, at baseline and before intervention, the I-FKT arm showed higher malaria parasite prevalence by RDT than the NI-FKT arm (aOR = 1.23; 95% CI [1.12-1.35]). Despite this difference at baseline, the parasite prevalence using RDT for detection was lower in the I-FKT than NI-FLT at endline (aOR=0.84; 95% CI: [0.74-0.96]), demonstrating the efficacy of the intervention. In the I-FKT, the parasite prevalence by RDT decreased from 8.0 to 4.7% for the overall population. The Difference-in-Difference (DiD) analysis for all people screened in either the baseline or endline surveys showed no difference for the overall population. However, the DiD analysis by age group demonstrated a marked difference for the children below 15 years of age, with a higher age group effect for children < 5 and between 5 to 14 years of age. This result highlights the importance of implementing malaria case control management in young and older children, such as adolescents.

Most of the project was conducted in remote areas. Thus, certain issues were encountered, mostly during the intervention period. It was not easy for the supervisors and CRAs to reach the fokontany due to rainfall. It was necessary to increase the number of supervisors from four to six for the 11 FKT. Also, it was necessary to add CHWs in areas where the hamlets were scattered or those for which the number of inhabitants per fokontany was higher than 1,000 residents. In total, 26 CHWs worked on the project instead of the 22 initially planned. In addition, there was a decrease in the number of study participants in the endline survey relative to that at baseline for both arms. One explanation is that it took place during the clove harvest period and some participants were not reachable. However, there were also a number of refusals after the baseline survey and the follow-up to contribute to the endline survey, with a lack of motivation of the CHWs to perform second or third visits to find the study participants or to motivate them to continue to contribute to the study. Most of the population had a low educational level and the sensitization activity could have perhaps been reinforced to convince them to contribute to the active screening.

This study allowed determination of the prevalence of anemia in pregnant women. At baseline, 57% of non-pregnant women of childbearing age had anemia, including 3% classified as severe (WHO definition). By the endline, the proportion dropped to 32%. For women with a positive malaria RDT, 73% presented with anemia at baseline and 51% at endline. We cannot draw any conclusions concerning the direct effect of the intervention on the anemia prevalence because we did not ask about nutrition, the notion of hemorrhage, or the presence of sickle cell disease, which are also important causes of anemia. However, the fact that there was no out-of-stock period for RDT and treatment during the study might have improved the access to malaria care of all participants, including the women of childbearing age.

Impact: The proactive community-based case management of malaria cases by CHWs for all age groups has already shown its efficacy in programmatic approaches in Senegal and Mali. For the first time in Madagascar, community-based case management was performed through a randomized cluster trial in a malaria-endemic rural area.

Our approach revealed an important advantage for children under the age of 15 years in the study area. This result highlights the importance of malaria case management not only for young children, but also for older children and adolescents.

Subproject SP22: Community survey on healthcare-seeking determinants in pregnant women and children under five to better understand related behavior to propose strategy improvements

Key leaders: Dr Feno Rakotoarimanana & Dr Chiarella Mattern

Background: Maternal and child health remains a major public health issue at the international level. In Madagascar, the population is continuing to grow (2.8% annual growth rate), with most (80%) people living in the capital city. In addition, 90% of the population lives below the threshold of 2US$ per day. Maternal and child morbidity and mortality remain high. Accessibility to healthcare centers, the use of healthcare facilities, and the provision of healthcare services is still low.

Study objective: To identify determinants and barriers specific to the local context that limit or prevent the provision of healthcare services, thus restricting access to quality maternal and child healthcare.

Methodology: This study was carried out in 2018 in three districts (Manakara, Moramanga, and Antsohihy) supported by USAID through Mikolo and Mahefa Miaraka. Determinants of the use of healthcare (preventive and curative) during pregnancy, the use of healthcare facilities or trained healthcare personnel for childbirth, family planning (before and after pregnancy), neonatal care (0-28 days), preventive and curative care for children under five years of age were determined and analyzed for pregnant women and children under five years of age.

Through the use of combined (quantitative and qualitative) approaches involving various healthcare research disciplines (clinical epidemiology, healthcare economics, healthcare geomatics, socio-anthropology, and the use of existing data), our results show that the context in Madagascar is not very different from that of most African countries.

Results and discussion:

Prenatal monitoring and childbirth:

- Nearly 80% of women had at least one prenatal consultation during their pregnancy and 65% were in their 3rd trimester of pregnancy at the time of our visit. The proportions are not significantly different between districts. Quantitative results and qualitative data show that the first prenatal consultation (CPN in French) generally occurs around the second or third trimester. Among women who did complete a CPN, many said they planned to do so in the third trimester, in the weeks after our interview.

- The motivations cited for having at least one CPN demonstrate the desire to respond to: 1) the need for confirmation of the pregnancy (awareness of the pregnancy is up to that point limited to the classical so-called presumptive signs, such as menstrual arrest, frequently feeling tired after the arrest of menstruation, and nausea), a willingness to monitor the state of health of the woman and child, and 2) the need for information in preparation for childbirth. The qualitative study showed that CPNs are perceived by women to be opportunities for the treatment/prevention of diseases specific to pregnancy, which may be harmful to them or to the development of their babies and may complicate childbirth (malaria was cited as a complicating factor in particular).

- The quantitative study showed that women with at a least primary-school education or more are more likely to have at least one CPN compared to women with no education. A pregnancy that is desired also increases the chances of having a CPN. Although the qualitative study showed that geographic constraints were little cited by women for not having a CPN, the quantitative study showed that this factor influences the use of this service. Indeed, the remoteness of the household in relation to a CSB demonstrates a significant risk of not having a CPN. The qualitative study showed that having a CPN is the woman's decision, and can be influenced by her husband's entourage, her female entourage, and the grandmother of the child. Current norms may also influence the decision. These standards are based on values shared by all communities and thus individuals will attempt to comply with them.

- The qualitative data highlighted strategies used based on information received at the CPN to decide whether to have the delivery at home or in a healthcare facility: this involves determining whether the woman is likely to experience a delivery characterized as easy, “tsysaro-petry”, or difficult, “saro-petry”. In addition, a CPN is considered as a "pass through" by women who, if they are referred to CSBs during childbirth, will receive a better quality of care. In this sense, the grandmothers of the children of the three zones encourage women to have a CPN.

- The healthcare system does not appear to have sufficiently integrated the expectations of the population in the management of the CPN and childbirth (e.g., the discretion that should surround the pregnancy and the benefits of traditional treatments, such as massages during pregnancy). The wish for discretion concerning an early pregnancy, shared across all zones, is contrary to a prenatal consultation during the first trimester of gestation. This requirement may be seen as a potential opportunity to reveal the secret and thus as a source of danger for the woman.

- Our qualitative data highlighted the importance of how well the quality of the services and care met the women's expectations and preferences, such as: shorter waiting times, the quality of reception, etc. The appreciation of the quality of services offered by users clearly influences decisions on the use of such a service.

- Our qualitative results also showed that women perceive pregnancy neither as a condition nor a risk factor for morbidity, but rather a normal pathway for the reproductive function of women. The advent of a pregnancy therefore does not automatically trigger the use of medical monitoring services.

Contraceptive methods:

- Overall, 58% of women used contraception before the survey. The proportions were not statically different between districts. The chances of using contraceptive methods increase with age. Nearly one quarter of women started using contraceptive methods before the age of 18. The fact that women have a good perception of family planning increases the likelihood of their using contraceptive methods. Following an ancestral religion provides women less coercion and therefore more freedom of choice in using contraceptive methods. For women in couples, spousal support greatly increases the likelihood of using contraceptive methods.

- Our survey showed a generally limited knowledge of modern contraceptive methods. Few women mentioned natural methods in their responses (qualitative and quantitative studies). However, the questions asked were unambiguous and included both natural and modern methods. Concerning natural methods, women referred more to the uncontrolled spacing of their pregnancies.

- Women's and men's knowledge of modern contraception mostly consists of three methods: injection, birth control pills and implants. Condoms and other male contraceptive methods are little known and never used, contraception being considered as the woman's responsibility.

- Nonetheless, family planning is discussed within couples. However, the man can be either a motivating or blocking factor, and we have not identified a clear trend for either position.

- According to the qualitative study, the main channel of information concerning contraceptive methods is the entourage. Discussions revolve more specifically around the

side effects of various methods but little on the personal experience of individuals. This can lead to the circulation of numerous rumors. The quantitative study, on the other hand, showed that CSBs are the primary source of access to information and products.

- The most commonly used contraceptive method is injection. This method is considered to be convenient from a logistical perspective (requires only one trip to the CSB every three months), discreet (no need for the husband to be aware), and no side effects have been noted. It is noteworthy that the availability of contraceptive injection products is not mentioned in the "National Action Plan budgeted for family planning in Madagascar 2016-2020", which states that CAs can provide the following methods for family planning: natural contraceptives, condoms, pills, and spermicides, but it does not mention injectables. This point therefore remains to be explored.

- The reasons for or against the use of a contraceptive method are the same for men and women. Factors that impede their use are the idea that the child is a resource for the household and therefore the spacing of births must result from a "natural" or God-given process rather than a couple's decision. Young women have little interest in family planning methods. Few responses from participants mentioned sexually transmitted diseases.

<u>Vaccination:</u>

- The concept of prevention was a misunderstood and undervalued notion by the people interviewed. In their view, prevention appears to encompass primarily child cleanliness and hygiene practices (clothes, food, or drinks).

- Less than half of the infants were vaccinated from the first to ninth month. Most who were, had between 1 and 5 vaccinations. None of the mothers mentioned vaccination beyond the ninth month. Our results show great variability in vaccination practices between siblings. Information on vaccination is particularly difficult to collect because the vaccination records are often lost.

- Obstacles in reaching full adherence with the immunization schedule are numerous: 1) difficulties in defining a vaccine (similar to an injection of antibiotics for example), 2) unclear information available for mothers (confusion between mass vaccination and routine immunization, they do not know the name of each vaccine or the number of vaccines and doses, let alone what diseases they can protect against), 3) obstacles associated with the names of the vaccines (e.g. BCG = no reference to the name of the tuberculosis the vaccine covers), 4) the fact that a single injection can protect against several diseases at the same time. However, mothers and fathers declared that an injection is equivalent to vaccination against a disease.

- The generally accepted idea is that vaccines are used to "strengthen child health". There is no differentiation made between diseases. This vision leads to disappointment and discouragement with regard to immunization, because "even if a child is vaccinated, he or she continues to get sick".

- There is no actor at the family level that, according to the interviews, appears to influence mothers in their choice of vaccination. Instead, they appear to receive the information and be motivated either during prenatal consultations by the physician or during CHW awareness.

Reliance on child-care:

- The diseases most frequently cited by parents are the tazo, i.e., fever problems, respiratory problems, and diarrhea.

- The first reflex when a child falls ill is self-medication. The next step is the use of CHWs, followed by consultation within a healthcare facility. The passage from one stage to the next and the length of time that elapses depends on several factors, including the age of the child and the reputation to the healthcare facility (reputation of the head of the post, perception of the quality of services offered, reception, etc.). Warning signs interpreted by parents as revealing the need to rush to a healthcare facility include no improvement in the general state of health of the child, despite first intention treatment, high fever, loss of appetite, and alteration of the child's general condition.

- The generic term “fever” encompasses three different realities. Populations generally distinguish between (1) simple maninjy/manavy fever, not accompanied by other symptoms, which can be cured by infusions or paracetamol at home, (2) malaria (tazomoka), a fever accompanied by other symptoms, such as vomiting, but mostly identified by CHWs by mRDTs, and (3) severe malaria (tazomahery), which, according to the inhabitants, is a worsening of malaria that is not treated properly and requires switching to a CSB.

- The use of CHWs for the care of children is a totally common practice and they are considered to be a category of actors with the capacity to improve the health of children. However, the practice is still strongly assimilated with malaria diagnosis, treatment, and/or reference to a healthcare facility. Little mention was made of other diseases for which the use of CHW could be useful.

- As for childbirth, the grandmother plays a role both in decision-making (where the child is delivered) and in providing more practical help (e.g., financial aspect, going back and forth to the CSB for treatment), especially when mothers are single and dependent on the grandmother.

- The quality of the relationship between residents and healthcare staff plays a major role in the recourse to care. The availability of healthcare staff and materials can be a factor in de-motivating/demotivating the population to switch to a CSB.

Impact: This study has produced unpublished data on various aspects of maternal and child health. Concerning vaccination, it demonstrates the need for a more in-depth study on vaccine hesitancy. Concerning contraception, it has brought to light previously unknown practices at the central level (distribution of injection contraceptive methods at the community level), as well as contraceptive preferences and key individuals involved in

contraceptive choices, with a view to guiding future programs. The section focusing on the management of diseases in children demonstrates the indispensable role of community workers.

Subproject SP26: Assessing the etiologies of diarrheal and respiratory infections in children below five years of age

Key leaders: Dr Laurence Randrianasolo, Dr Iony Razanajatovo & Dr Norosoa Razanajatovo

Background: mRDTs are used to confirm malaria and are indicated for all cases of fever. The increasing number of negative mRDTs reveals a growing proportion of patients with fever who do not have malaria. Through a syndromic approach, patients can thus be classified into influenza-like illness (ILI), suspected arbovirus infection, or diarrhea.

Data obtained through the Fever Sentinel Surveillance network in 2017 showed that children under five years of age represented 30% (10,307/92,519) of outpatients, of which 11% (10,307/92,519) presented fever syndromes. ILI (52%) comprised the most observed cases, followed by diarrhea (12.5%), malaria (7.4%), and suspicion of arbovirus infection (0.1%) (Randrianasolo, L. 2010).

Malaria was reported to be the main cause of fever on the east coast, whereas diarrhea was more frequent in the north. More than 30% of fever syndrome remained undetermined.

Routine immunization activities and vaccination during mother and child health weeks were initiated in 1980. However, two outbreaks of VDPV (vaccine-derived poliovirus) occurred in the southwest and southern parts of Madagascar (2001-2002 and 2005). In October 2014, a case of AFP (acute flaccid paralysis) was reported in the Analalava district, in the Region of Sofia, situated in the north-west. Stool analysis revealed a PV1-discordant virus. Certain districts were then classified as high-risk zones for epidemic poliovirus, as the vaccination coverage was < 80%.

Study objective: To determine the etiologies associated with diarrhea and respiratory infections in children aged below five years of age to reinforce the detection capacity of the biological surveillance system and train physicians in biological specimen management.

Methodology: Nasopharyngeal samples were collected from patients presenting with ILI or severe acute respiratory infection at the Fever Sentinel Site between January 2018 and August 2019 to identify the etiology of respiratory infections in children under 5 years of age. Respiratory pathogens were detected using Fast Track Diagnostics technology, which that can detect 21 respiratory pathogens. Samples were also tested for RSV and rhinovirus by RT-PCR and seasonal influenza virus type A and B following the U.S. Center for Disease Control and Prevention (CDC) PCR protocols.
Stool samples and rectal swabs were collected from children under five years of age who presented with acute diarrhea at one of the Fever Sentinel Sites between August 2018 and August 2019 to identify the etiology of diarrheal infection. Viral and bacterial pathogens

were detected using FTLyo Viral Gastroenteritis & FTLyo Bacterial Enteropathogen kits (Fast Track Diagnostics Ltd, Malta) and protozoa were detected using a Solis Biodyne kit for simplex Entamoeba histolytica and an in-house duplex assay for *Cryptosporidium parvum* and *Giardia intestinalis*.

Results and discussion:

Etiology of respiratory infections: In total, 475 nasopharyngeal samples collected from January 2018 to December 2018 from children aged less than five years were tested. Based on the Fast Track Diagnostic tests, 87.4 % of tested samples contained at least one pathogen. Among the positive samples, 12.6% contained one pathogen, whereas two or more pathogens were identified in 74.7% of samples. Viral and bacterial pathogens were detected in 82.1% and 72.2% in tested samples, respectively. *Haemophilus influenzae b* (Hib) was the most detected pathogen, followed by enteroviruses, rhinovirus, influenza type A, *Streptococcus aureus*, *Mycoplasma pneumoniae*, RSV, human metapneumovirus, influenza virus type B, parainfluenza virus type 3, and parechovirus.

Nasopharyngeal samples collected from January to August 2019 were tested for the presence of respiratory viruses (Influenza, RSV, and rhinovirus). Respiratory viruses were detected in 56.0% of tested samples, with predominant detection of influenza viruses (32.4%), followed by RSV (23.5%), and rhinovirus (6%). RSV was more frequently detected in patients younger than six months (43.1%, 56/130) compared to influenza (19.2%, 25/130). In contrast, influenza was prevalent in patients aged more than two years (48.1%).

Etiology of diarrheal infections: In total, 321 stool samples or rectal swabs were collected on a routine basis between August 2018 and august 2019 from children aged less than five years. Children between 6 and 23 months of age were by far the patients consulting most for febrile or acute diarrhea, followed by those between 24 to 59 months of age. Among the viral pathogens detected, rotavirus represented the highest proportion, at 28.3%, followed by Astrovirus (21%), Adenovirus (16%), Norovirus GII (10%). Norovirus GI and Sapovirus were detected in 6% of samples tested. Among bacteria, Shigella was the most commonly identified, at 39%, followed by *Campylobacter coli*, detected in 19% of samples. The prevalence of *Salmonella* spp., *Escherichia coli*, and *Yersinia enterolitica* was between 0 and 5%. Protozoa were also detected, with *Giardia intestinalis* identified in 30% of tested samples, *Cryptosporidium parvum* present in 12%, and *Entamoeba histolytica* in 7%.

Impact: This study describes the etiologies associated with respiratory and diarrhea infections in children under the age of five in four regions of Madagascar. Influenza viruses and RSV were among the most commonly detected pathogens in acute respiratory illnesses. Hib was detected at high rates, despite routine immunization that includes a vaccine against Hib. The results highlight the need to implement surveillance of EVs, in particular EV68, as this virus can cause outbreaks of pneumonia or meningitis. This study also highlights the need for the development of an effective RSV vaccine.

The detection of Rotavirus at high frequency in children with acute diarrhea highlights the importance of good vaccine coverage to avoid outbreaks. In this study, *Shigella spp.* was the most detected bacterial pathogen. This observation warrants further surveillance due to the emergence of multidrug resistant strains of bacteria throughout Madagascar.

Subproject SP31: Qualitative assessment of prematurity and the Kangaroo Mother Care method in Madagascar

Key leader: Dr Chiarella Mattern

Background: In Madagascar, 14% of children are estimated to be born prematurely, which exposes them to a higher risk of morbidity and mortality. Results from a longitudinal study on bacterial infection and antibiotic resistance to them among young children in low-income countries (BIRDY 1) showed a substantial prevalence of low gestational maturity of young children in Madagascar. The goal of this qualitative study was to complement the second phase of BIRDY to understand the determinants of premature births in Madagascar to propose recommendations for the management of premature births and the implementation of the KMC (Kangaroo Mother Care) method.

Study objective: To identify and analyze the knowledge, attitudes, and management of preterm children among mothers, grandmothers, and healthcare workers in communities in three districts (Antananarivo, Moramanga, and Manakara) and describe the practice of the KMC method to identify difficulties encountered by healthcare workers and mothers at the KMC unit in Antananarivo.

Methodology: The study protocol was submitted for review to the MoPH Institutional Review Board in December 2018. The study was conducted in two parts. In the first, a survey was conducted on the management of premature births in communities in Antananarivo, Moramanga, and Manakara. Focus groups and semi-structured interviews were used. The second phase focused on the KMC unit in Antananarivo. Direct observations of the practices at the KMC unit, semi-structured interviews of healthcare professionals working the then KMC unit, and observations and semi-structured interviews of mothers were performed.

Results and discussion: The study protocol was approved by the MoPH in February 2019. Focus-group discussions and semi-structured interviews of mothers to identify the knowledge, attitudes, and management of preterm children within the community showed that most women have critical living situations (working conditions and marital conflicts) which affected their pregnancy, poor living conditions make it difficult for pregnant women to follow health recommendations given during the pregnancy, dating the delivery period is difficult, some healthcare professionals lack knowledge and training concerning preterm births, healthcare professionals do not share sufficient detailed information about the health and medications of the mothers and children, and grandmothers of the children do not have sufficient information about the risk of preterm birth and what they should do while caring for a pregnant woman.

Concerning the practice of the KMC method at the Kangaroo unit in Antananarivo, results showed that mothers, as well as healthcare professionals, have a good opinion about KMC, although mothers carried the baby much less as soon as they were back home, given their daily tasks and the fact that there is no supervision 24h/24. During our observation of the practice of KMC, mothers were often unavailable at the beginning of KMC due to health problems that affected breastfeeding (a very important part of the KMC method) and the process of acquiring the practice of the KMC method by the mothers. Social and financial problems surrounding the use of the KMC method appear to have an impact on whether the mother practices the method and stays in the KMC unit. Space and medical resources were limiting factors at the KMC unit, as this is the only KMC unit and the high demand was high.

Impact: Very little data concerning preterm births are available in Madagascar (risk factors, causes, medical care, etc.). This was the first socio-anthropological study to examine preterm births in Madagascar. Various socio-cultural determinants associated with preterm births were identified. The results can be used to improve the medical care provided to preterm children and improve the KMC method and eventually expand its practice in other parts of Madagascar.

WP3. PLAGUE

Plague is an arthropod-borne zoonotic disease of which the natural reservoir is rodents but periodically causes human infection with the classical clinical presentation of bubonic plague. It is still endemic in approximately 25 countries in the world, but currently 95% of human cases (between 500 and 600 annually) are declared in only two regions of the world: the Ituri district in the North-East of the Democratic Republic of Congo and in Madagascar. In Madagascar, most cases occur annually in specific endemic areas of the Central Highlands, with a clear seasonal pattern (between October and April). Limited outbreaks of plague occur periodically, including occasional pneumonic plague outbreaks and isolated cases. This clinical form is more severe than the classical bubonic form and is potentially transmissible between humans. Between August and November 2017 an unprecedented outbreak of pneumonic plague hit Madagascar, affecting mainly the cities of Antananarivo and Toamasina, with 1,878 clinically suspected human cases recorded. This event serves as a reminder that plague is still a serious threat with an outstanding potential to disrupt human societies, as evident from historical accounts.

The IPM has a significant track record of plague surveillance and research since its foundation in 1898, at around the time that plague was introduced to Madagascar. Indeed, most of the seminal and current knowledge of plague and its control measures have been produced here. Currently, the IPM hosts one of the four plague reference laboratories in the world. With the resurgence of an outbreak of urban plague, it became clear that further support for this unit would be necessary, and, in general, for plague surveillance. As a result, a further work-package was added to the USAID Grant: first, to support the emergency response to the outbreak; second, to strengthen plague surveillance and monitor prevention measures to avoid similar events in the future; and third, to establish post-outbreak research to better understand the epidemic.

Subproject SP23: Support of the 2017 plague epidemic response

Key leaders: Dr Rindra Randremanana & Dr Minoarisoa Rajerison

Background: The Epidemiology Unit at the IPM assisted the Central Laboratory for Plague (CLP) and the DVSSE_R (Direction de la Veille Sanitaire, de la Surveillance Epidémiologique et de Riposte) at the Malagasy MoPH, to complete the official the national database of plague notifications for surveillance during the 2017 plague epidemic. The magnitude, location (2 main urban areas: Antananarivo and Toamasina), and clinical form (mainly pulmonary) of the epidemic led the IPM to dedicate more human resources and prioritize (or de-prioritize) certain activities.

Study objective: To reinforce the 2017 plague epidemic response.

Methodology: At the beginning of the plague epidemic season (September 2017), 30% of the staff at the epidemiology unit of the IPM were assigned various tasks within the plague

epidemiological surveillance team: management of the plague database (data entering and cleaning), data analysis and interpretation, reporting, and clinical research protocol preparation. They attended numerous meetings to share their expertise in epidemic response and surveillance.

Results and discussion:

Notification cases

From November 1, 2017 to July 31, 2018, 756 observations from plague notification forms were entered into the database. Among them, 575 were pneumonic plague, 149 were bubonic plague, and 32 were unknown. Among the patients with pneumonic plague, nine were confirmed cases (suspected cases with both a positive RDT and PCR or *Yersinia pestis* isolated from the collected samples or seroconversion) and 39 were classified as probable (suspected cases with positive RDT of plague or a positive PCR test). The case-fatality rate for pneumonic plague was 77.7% (7/9) for confirmed cases and 7.7% for probable cases. The confirmed cases were mainly from districts outside the capital, such as Ambalavao (n = 2), Fandriana (n = 2). The remaining confirmed cases were from Ankazobe, Miarinarivo, Faratsiho, and Andramasina. For the probable cases, 46% (18/39) were mainly from Antananarivo city and the "Grand Antananarivo" (28%).

Among those diagnosed as bubonic plague, 55 were confirmed cases (17 deaths, case-fatality rate for confirmed bubonic plague: 31.5%) and 16 were probable cases. The confirmed bubonic plague cases were mainly from plague endemic areas, such as the Ambalavao (n = 9), Tsiroanomandidy (n = 8), and Ambositra (n = 8) districts.

Approximately 44.4% (n = 336) of notified cases during this period came from Antananarivo city, 22% from the "Grand Antananarivo", and the remaining cases from outside the Analamanga region.

The database was transferred on a daily basis to the DVSSE_R until November 2017. From December 2017 to July 2018, the transfer was performed on a weekly basis.

GIS activities

GIS activities covered the period from August 2017 to December 2017.

To enable the mapping of notified cases, the GIS team worked on cleaning up all data related to the place of residence of the notified cases and the healthcare centers where they were notified. Among the 2,575 recorded cases in the database, 93% were geocoded at the district and commune level and 63% at the "*fokontany*" level. Among 453 healthcare centers (HCCs) that performed case notification, 97% were geocoded at the district level and 42.2% geocoded at the "*fokontany*" level. The absence of geocoding of the remaining proportion was due to wrong addresses or missing data concerning the location. Since January 2018, a program has been developed to obtain the complete address of notified cases by checking the OCHA code directly via a web application to obtain the corresponding code before data entry.

Community health education (Health and Social Sciences Group, SaSS)

During the 2017 plague outbreak period, one member of the Health and Social Sciences Group served as the focal point for the IPM within the communication committee for plague response by:

- actively participating in all meetings of the communication committee
- being part of the coordination committee for communication
- participating in actions organized by the media and press subcommittee (advocacy of the press leaders, training of the district's journalists, production of video-reports of former victims of plague who recovered, preparation of a TV program on the plague)

This individual was also in charge of ensuring the communication flow and collaboration among groups of anthropologists and researchers working in various institutions, such as the IPM, WHO, and UNICEF.

Impact: The Epidemiology unit at the IPM: i) successfully assisted the Central Laboratory for Plague and the DVSSE to complete the official national surveillance database of plague notifications, ii) undertook real-time mapping of notified cases to monitor the epidemic, and iii) supported communication by participating in the Communication Committee for plague response and enabled communication between the IPM, WHO and UNICEF.

Subproject SP24: Support of the Central Laboratory - 2017 plague epidemic response

Key leader: Dr Minoarisoa Rajerison

Background: Madagascar accounts for 75% of global plague cases reported to the WHO, with an annual incidence of 200 to 700 suspected cases (mainly bubonic plague). In 2017, a pneumonic plague epidemic of unusual size occurred. The extent of this epidemic provides a unique opportunity to better understand the epidemiology of pneumonic plagues, particularly in urban settings.

Methodology: Clinically suspected plague cases were notified to the CLP at the IPM (Antananarivo, Madagascar), where biological samples were tested. Based on cases recorded between August 1 and November 26, 2017, we assessed the epidemiological characteristics of this epidemic. Cases were classified as suspected, probable, or confirmed based on the results of three types of diagnostic tests (RDT, molecular methods, and culture) according to 2006 WHO recommendations.

Results and discussion: In total, 2,414 clinically suspected plague cases were reported, including 1,878 (78%) of pneumonic plague, 395 (16%) of bubonic plague, one (< 1%) of septicemia, and 140 (6%) with an unspecified clinical form. Among them, 386 (21%) of the 1,878 notified cases of pneumonic plague were probable and 32 (2%) were confirmed. Among the 395 notified bubonic plague cases, 73 (18%) were probable and 66 (17%) were confirmed. The case fatality ratio was higher among confirmed cases (8 [25%] of 32 cases) than probable

(27 [8%] of 360 cases) or suspected pneumonic plague cases (74 [5%] of 1,358 cases) and a similar trend was observed for bubonic plague cases (16 [24%] of 66 confirmed cases, 4 [6%] of 68 probable cases, and 6 [2%] of 243 suspected cases). Among the 418 confirmed or probable pneumonic plague cases, 351 (84%) were concentrated in Antananarivo, the capital city, and Toamasina, the main seaport. All 50 isolated *Yersinia pestis* strains were susceptible to the tested antibiotics.

In this study, we describe the epidemiology of the 2017 plague epidemic in Madagascar. This predominantly urban plague epidemic was characterized by a large number of notifications in two major urban areas, with a quarter of cases classified as confirmed or probable, and an unusually high proportion of pneumonic forms, with only 23% having one or more positive laboratory tests. This study provides a unique opportunity to better understand the epidemiological characteristics of pneumonic plague in a densely populated urban setting. The outbreak also illustrates the many challenges associated with the control of plague in Madagascar. Lessons about clinical and biological diagnosis, case definition, surveillance, and the logistical management of the response identified in this epidemic are crucial to improve the response to future plague outbreaks.

The epidemic was characterized by exceptionally large numbers of pneumonic plague case notifications (1,878 in 2017 *vs* 83 per year on average in 2010–15), although the number of notified bubonic plague cases remained similar to that of recent years. The rapid increase in notified cases, particularly of the pneumonic form, at the end of September 2017, prompted a large national and international multisectoral response, the creation of a national emergency task force, and a joint response plan to curtail the epidemic. The urban nature of the epidemic, its multiple foci, and the potential for international human-to-human spread, as well as the potentially high lethality, required rapid and sustained multipronged efforts. The outbreak also had a substantial impact on society (e.g., school closure) and travel and trade for Madagascar (e.g., implementation of airport screening measures—some airlines cancelled flights). The large number of suspected cases was a major hurdle for logistical management of the various aspects of the response.

This outbreak was the first time that the CLP had received such a large number of pneumonic plague samples, which raised several challenges for the laboratory confirmation of cases. The tests initially used by the CLP had mainly been performed on bubonic plague samples and their performance on primary pneumonic plague samples had not been evaluated. Testing the presence of *Y pestis* is much more challenging for pneumonic plague because of the quality of sputum samples and the contamination of samples by the commensal flora of the upper respiratory tract. For example, the insufficient specificity of conventional *pla* PCR on pneumonic plague samples led to rapid implementation of improved molecular diagnostics. Samples collected before November 3, 2017, were retrospectively retested in November and December 2017 using the upgraded molecular biology techniques. The higher specificity of these techniques led to a decrease in the final number of confirmed or probable cases relative to those presented in previous reports during the outbreak, particularly for pneumonic plague cases.

Therefore, the magnitude of the pneumonic plague epidemic is likely to have been smaller than suggested by the number of notifications, as only 23% of notified pneumonic plague cases had more than one positive laboratory test, with laboratory results available for more than 99% of cases. The spatial extent of the pneumonic plague epidemic appears to have been relatively restricted, with 84% of the confirmed or probable pneumonic plague cases observed in the initial two main urban transmission sites (Antananarivo area and Toamasina). With a doubling time of five days, the growth in confirmed or probable pneumonic plague cases was rapid, but part of the observed growth may have been due to increased reporting due to enhanced contact tracing and an increase in public awareness. Factors that could explain the over-reporting of pneumonic plague cases include the limited clinical experience in newly affected areas (pneumonic plague is rare and few clinicians in Madagascar had had first-hand experience of it) and the difficulty of clinical diagnosis in a context in which respiratory signs can be caused by other circulating pathogens (e.g., a concomitant outbreak of bronchiolitis among children). Indeed, patients with pneumonic plague initially present with mostly non-specific upper respiratory symptoms, such as cough, fever, and headache, and differential diagnosis is therefore difficult on clinical grounds, particularly in the early stages of the disease. Certain similarities to this outbreak can be seen in an outbreak of pneumonic plague in India in 1994, which resulted in more than 6,000 notifications for less than 300 confirmed or probable cases. During the Indian epidemic, experts recommended that suspected cases with negative biological test results remain classified as suspected. Overall, more than 99% of suspected cases tested negative by both RDTs and PCR (with a culture that was either negative or not performed). Investigating such negative cases more thoroughly in future outbreaks and revising guidelines accordingly may help better to better characterize the true magnitude of plague outbreaks.

In a context such as this, in which confirmatory diagnostics are challenging, it is still difficult to precisely quantify the prevalence of plague among notified cases. For example, newly implemented RT-PCR that targets two genes is expected to be highly specific but could have insufficient sensitivity. Therefore, the true prevalence of plague in notified pneumonic plague cases is likely to lie somewhere between that of confirmed and probable cases, justifying why we performed our analyses on confirmed and confirmed or probable cases as a joint group. This theory was corroborated by the fact that the numbers for demographic, clinical, and epidemiological characteristics of probable cases often fell between those for the confirmed and suspected cases.

The Case Fatality Rate (CFR) of suspected (5%), probable (8%), and confirmed (25%) pneumonic plague cases differed markedly. These differences may be due to several factors, including the proportion of false positives among probable cases, a lower probability of being diagnosed as a confirmed case among non-deaths, as sputum samples have a lower yield than do lung or liver samples, which are only taken from dead individuals, and early and frequent antibiotic use (because of frequent self-medication facilitated by the availability of antibiotics without a prescription) and better clinical care, which may both reduce the probability of confirmation (i.e., hence cases are probable) and increase the likelihood of survival (hence lower CFR). These factors may also partially explain why the CFR did not substantially change with the response. Better access to healthcare and more intensive use of antibiotics in cities

(that were predominantly affected by pneumonic plague) may partially explain why the CFR of pneumonic plague cases was lower in cities than in the endemic zone and why pneumonic plague cases were not more severe than bubonic plague cases, as is typically observed. The CFR was particularly high in cases with an unspecified clinical form, most of whom died before the history of symptoms could be accurately reported. No nosocomial cases were identified using the notification forms.

Serology was not performed because the collection of blood samples was restricted by logistical constraints and was not recommended by the Plague National Control Program in this epidemic context.

We can only speculate about the factors that led to such an unprecedented outbreak. The early start of the outbreak may have been induced by changes in the demography or behavior of the reservoir (potentially due to climatic or ecological variations), which could have increased the risk of contact with humans. Detection of pneumonic plague cases can be more challenging in the middle of the austral winter because of the concomitant circulation of other respiratory diseases with similar symptoms. Once plague reaches multiple locations, including urban centers with high population densities, management and control of the epidemic becomes much more challenging. The lower median age for bubonic plague cases could be explained by behavioral factors: young adults are more highly involved in agricultural activities, exposing them to contact with rodents and fleas, and children spend more time close to the floor than do adults, leading to greater exposure to flea bites.

There were several limitations to this study. The samples and data used were collected during the response to a major epidemic and should be interpreted in this context. For example, information on pre-examination treatment was collected as free text and the absence of treatment could not be distinguished from missing information. Acquiring information from severely ill cases is difficult and the quality of collected data may be affected by outcome, potentially leading to reporting biases.

Despite these challenges, our study provides invaluable information about the characteristics, epidemiology, and transmission dynamics of pneumonic plague. This epidemic illustrates the difficulty in adapting medical and public health responses during an epidemic of unusual magnitude and clinical form in predominantly urban areas. In such an emergency context, national and international multidisciplinary mobilization is important to support real-time surveillance capacity, improved microbiological testing, community sensitization, and the protection of healthcare workers. Structures and strengthened surveillance mechanisms put in place during the epidemic now need to be optimized to strengthen national and international response capacities in case of another urban outbreak. Additionally, multidisciplinary research programs to improve diagnostic algorithms, alternatives to aminoglycoside-based treatment, immune response mechanisms in humans, and studies disentangling the causes for re-emergence are required.

Impact: Overall, this epidemic confirmed the significant public health risk of the re-emergence of pneumonic plague in urban areas and its potential for rapid expansion. Lessons learned

from this epidemic will form the basis for improved plague investigation and response efforts in Madagascar and beyond.

Subproject SP25: Assessment of the risk of drug adverse events, knowledge of the disease, and the perception of transmission risk during the plague epidemic

Key leader: Dr Rindra Randremanana

Background: According to the national surveillance system for plague in Madagascar, 2,414 cases were notified during the 2017 Plague Epidemic, of which 78% was pneumonic plague. Most cases were notified in Antananarivo, the capital city, and Toamasina, the main seaport. The epidemic period was defined as August 1 to November 26, 2017. The health authorities and their technical partners faced a number of difficulties in controlling the epidemic and international support was needed.

Quantitative and qualitative studies were included in this project.

Quantitative study

Study objective: To better understand the epidemic by conducting a post-epidemic survey in Antananarivo to assess the true magnitude of the epidemic using serology and estimate the potential adverse side effects due to the use of high doses of Streptomycin.

Methodology: The clinical and serological characteristics of plague survivors and household members were determined. The risk of adverse side effects of Streptomycin, mainly ototoxicity and renal insufficiency were estimated. A seroprevalence study was conducted on plague survivors living in Antananarivo who notified the Central Plague Laboratory located in the Plague Unit of the IPM from March to July 2018. The household members were also investigated, together with a sample of the general population of Antananarivo (sampled among the consultants or their family members attending the anti-rabies care clinic (CTAR) of the IPM for post-exposure vaccination). For serology, in addition to the IgG anti-F1 ELISA method, a new Multiplex (using Luminex Magpix technology) was developed with several *Yersinia pestis* antigens for IgG by the Infectious Diseases Immunology Unit in collaboration with the Plague Unit at the IPM. A nested age-matched exposed/non exposed to Streptomycin study was performed. Additional biological testing was performed, such as the measurement of creatinine levels and audiometric testing of subjects included in the nested study.

Results and discussion: Among 312 plague patients identified in the database, 115 were included (7 confirmed, 61 probable, and 47 suspect cases). In addition to these 115 cases, 259 household contacts and 200 subjects from the general population were included. For the nested age-matched exposed/non exposed to Streptomycin study, 51 pairs were analyzed for renal function and 36 for audiometric testing. The vast majority of plague survivors (98%) recovered, and no severe sequelae were observed.

Serological results (anti-F1 IgG detection) during the convalescent phase after disease onset were all negative by ELISA. In total, 457 samples were tested using the multiplex technique.

With this new method, we found eight subjects who were positive for anti-F1 IgG (2 probable cases, 5 contacts, and 1 from the general population). No renal failure was observed in subjects treated with Streptomycin. There was no statistically significant difference in the audiometric testing between the exposed and non-exposed groups. However, a few symptoms (vertigo, equilibrium disorder) were reported by the plague survivors at the time of the Streptomycin treatment. Vertigo appears to have persisted for a few months after the disease.

The epidemic context in urban areas made recruitment difficult; there were 22% refusals and 28% false addresses. Based on these results, it is recommended to explore the four humoral and cellular immune responses during the acute phase after disease onset and to use the multiplex technique. The lack of differences between subjects exposed and non-exposed to Streptomycin for renal function and audiometric testing may be due to the lack of power of the study or to the fact that the high doses of Streptomycin were taken for only a couple of days for plague and the subjects were included in the study a few months after disease onset. However, clinical symptoms, such as vertigo, were reported by the patients.

Impact: This study highlights the need for a coordinated response among partners and better strategic planning. It also helped us to better understand the difficulties encountered in conducting post-epidemic research for a disease with high stigmatization, such as plague and further underlined the need to introduce the collection of pharmacovigilance data during and immediately after the epidemic period. Finally, it helped us to consider new research pathways to better understand and fight plague.

Qualitative study

Study objective: To assess knowledge on the disease and the perception of the risk of transmission in the post-epidemic period of communities, patients, and healthcare workers in Antananarivo using a socio-anthropological survey.

Methodology: The protocol was approved by the Ethics Committee (N°012 MSANP/CERBM). The survey was conducted between February and June 2018 in the field and data analyses were performed until December 2018. The survey was led by the Health and Social Sciences Group (SaSS) of the Epidemiology and Clinical Research Unit at the IPM. The survey covered three themes. The first focused on the experiences of care during the epidemic and the difficulties encountered during the clinical management for hospitalized patients. Fourteen healthcare professionals working in the main plague care center during the 2017 outbreak, as well as 31 recovered patients, were interviewed using semi-directive questionnaires. The second theme focused on the perceptions and knowledge of plague in the general population. Ten focus groups composed of 46 individuals, in total, were conducted using the photovoice method. Thus, participants had to take three pictures that responded to the following question: *"What reminds you of plague in your environment?"*. Transcription and translation were performed for all the interviews and focus groups and the data analyzed by topic. The third theme focused on the analysis of print and social media data during the 2017 plague outbreak. These data came from "information watch" and were collected by the official "communication commission" coordinated by UNICEF between October and November 2017

during the epidemic. The information and rumors circulating about the disease reflect the population's understanding and perceptions of it. The analysis was performed by theme and observed over time to elucidate the evolution of the social constructs concerning the disease.

Results and discussion: The results of this survey showed that plague is a known disease, but that its characteristics remain unclear for most of the population, including its causes, its transmission pattern, and the distinction between pulmonary and bubonic plague. Plague was systematically associated with a lack of hygiene and poverty, which exacerbated the feeling of shame and stigmatization of sick people by those around them. The progressive awareness that anyone can be infected by the plague comes up against the social representations of the disease, leading to fear. This perception often leads to misunderstanding and refusal of the diagnosis by the patients, encouraging some of them to run away from hospitals. These shortcomings and the general lack of information from the authorities during the epidemic maintained a sense of panic and reinforced fear. They also promoted the emergence and circulation of rumors and conspiracy theories through the mass and social media. These rumors, as well as fear and misrepresentations of the disease, influenced the behaviors that were adopted throughout the epidemic (e.g., self-medication, caregivers' behaviors to prevent giving the disease to their family) and have had an impact on public health measures.

Additional data were collected between October 2018 and January 2019 to consolidate the main findings by interviewing healthcare workers in another treatment center. A detailed report is under preparation.

Impact: This study highlights the need to consider the social dimensions of such an outbreak and the written media that accompanied the health crisis of the 2017 plague epidemic to better understand their possible impact on public health measures and respond appropriately.

Subproject SP27: Sentinel surveillance of plague risk indicators (rodents and fleas) in Madagascar

Key leaders: Dr Soanandrasana Rahelinirina, Dr Mireille Harimalala, & Jerry Rakotoniaina

Background: One of the most important activities for plague prevention is plague vector and mammal host surveillance. Rodent infestation and increases in flea density above defined thresholds are important risk factors for murine epizootic diseases and, eventually, human epidemics due to contact between rats, fleas, and humans. Dead rat surveillance and carnivore sentinel and flea abundance records provide an indication on the circulation of plague and the risk of human transmission. In addition, the sensitivity of fleas to insecticides needs to be monitored because multiple resistance is suspected in flea populations. Rodent-flea surveillance and control activities, particularly in plague endemic districts, are defined in the national strategic plan to fight against plague. The IPM Medical Entomology Unit and Plague Unit scientifically and operationally support the Plague Service of the MoPH for these activities.

Plague risk indicators include rat die offs (epizootic disease), infection of sentinel animals, rat and rat flea density (flea index and specific index), the circulation of *Yersinia pestis* among rodents and fleas, off-host flea density, the susceptibility status of fleas to insecticides (lab tests), and the training of community healthcare agents.

Study objective: To identify plague risk indicators according to recommendations made during the workshop held in Mantasoa, from July 17 to 20, 2018, and to update the national strategy for the prevention and control of plague in Madagascar.

Methodology: Small mammals were captured from December 2018 to May 2019 using live traps in five districts (15 sites) ranging in plague endemicity from none to active foci. For each animal captured, the fleas were removed, counted, and identified to determine the flea index (average number of fleas per host) and to detect *Y. pestis* DNA using PCR. We also collected blood (sera) to detect IgG anti-F1 *Y. pestis* antibodies by ELISA and spleen for testing *Y. pestis* F1 antigen using RDTs. In addition, we collected free-living fleas in households using candle traps. Unusual rodent die-offs that characterize plague epizootics were noted. Sera collected from 16 dogs in small mammal collection sites were tested by ELISA for *Y. pestis* antibodies.

Results and discussion: Among the 15 sites, 557 small mammals were captured, consisting of *Rattus rattus*, *R. norvegicus*, *Mus musculus*, and *Suncus murinus*. Among 557 spleens, 3/166 (1.8%) from an active focus, and 3/126 (2.4%) from a focus with no reports of human plague since 2015, were positive by RDT. Sera from 18/166 (10.8%) small mammals from an active focus were IgG anti-F1 positive. In total, 1,547 fleas were collected from small mammals and *Xenopsylla cheopis* was the predominant rat flea. The flea index ranged from 0.8 to 6.9 per animal, the highest of which was from a plague-free area. In total, 1,833 fleas were collected from households; *Pulex irritans* was the predominant species. None of the 1,320 rat and household fleas tested by PCR was *Y. pestis* positive. No rodent die-offs were reported at any site. Of the 16 dogs sampled, one from an ancient plague focus, was IgG anti-F1 positive.

In total, 25 insecticide tests could be performed in the laboratory using 15 flea populations collected from the field. Ten, 8, and 7 tests could be performed using 1% fenitrothion, 0.75% permethrin, and 0.05% deltamethrin, respectively. Most of the flea populations were resistant to the three active ingredients, whereas only three population were susceptible to 1% fenitrothion.

Impact: *Y. pestis* circulates at low rates in animal populations in active, inactive, and ancient plague foci. Our findings highlight the necessity to continuously follow adopted plague control measures to avoid exposure to infected rodents and their fleas.

Flea populations exhibited resistance to tested insecticides. Resistance may be related to insecticide use for plague responses, but it may also be associated with the unregulated use of these chemicals (agriculture). Fenitrothion may still be a useful prevention tool in sites where fleas remain susceptible.

Subproject SP28: Lab processing of human samples during the 2018-2019 plague season (October 3rd, 2018 to September 30th, 2019)

Key leaders: Dr Beza Ramasindrazana, Dr Voahangy Andrianaivoarimanana, & Dr Jerry Rakotoniaina

Background: Laboratories play crucial roles in terms of ensuring and improving the rapid understanding of the origin of outbreaks. They provide required information about plague circulation to rapidly tackle the extension of outbreaks. The Central Laboratory for Plague (CLP) of the MoPH is located at the Plague Unit of the IPM. Thus, the CLP actively participates in plague confirmation at the national, regional, and global level. The CLP has high level expertise for this pathogen in terms of diagnosis and research capacity, which is a key component to support the surveillance and response to the disease.

If plague is suspected, clinical specimens should be collected quickly, safely, and appropriately. For the confirmation at the CLP, a combination of biological tests (RDTs, molecular biology, bacteriology, and serology) is needed, as none of them alone have sufficient sensitivity and specificity to provide an accurate diagnosis (particularly for pneumonic plague). In addition, the Laboratory is currently producing RDTs, which are used for plague diagnosis.

For each *Y. pestis* strain isolated by bacteriology, susceptibility testing to the antibiotic recommended by the Plague National Control Program (PNCP) and other antimicrobial agents has been undertaken. If *Y. pestis* is resistant to one or more of the tested antibiotics, efficient therapy or prophylactic therapy is determined. At the moment, however, all *Y. pestis* strains are susceptible to the tested antibiotics.

Study objective: To support the Plague Unit for the laboratory processing of human samples

Methodology:

- Pre-season training and supervision missions on the use of RDT and plague notification were first conducted. Emphasis was placed on sampling, rapid and secure transportation (triple packaging), and selective use of RDTs (in compliance with the National guidelines, RDTs not to be used on primary pneumonic plague). These activities were conducted in collaboration with the Plague Service and the DVSSE_R of the MoPH. The supervision missions were adapted according to the onset of plague cases in the endemic zones.

- A guide for triple packaging and distribution to healthcare centers within plague endemic-foci was edited to ensure the secure transportation of samples to Antananarivo and other healthcare facilities and contracts were signed with a national transportation company.

- All suspected plague cases reported to the CLP of the IPM were confirmed by RDTs, conventional PCR, real-time PCR, and bacteriological culture.

- Investigation of the outbreak and case confirmation were conducted (with the mobile laboratory, when available).

- Three additional individuals were hired to reinforce the IPM plague team: one lab junior technician; one lab senior technician, and one data manager.

Results and discussion: During the 2018-2019 plague season (01/08/2018 – 31/07/2019), the number of notified plague cases decreased considerably relative to 2017-2018 (270 vs. 2,708) but was comparable to previous plague seasons. The confirmation rate (among notified cases accompanied by biological samples) was better than for 2017-2018 (40.22% vs. 5.08%). This season was highlighted by improvements of the confirmation rate after the implementation of molecular biology methods (qPCR and conventional PCR) since the 2017 pneumonic plague outbreak and which constitutes a confirmatory test when combined with a RDT. The duration of sample processing using molecular methods was greatly improved, with 0.3 day vs. 33.9 days (this time interval refers to the time elapsed between sample reception and the availability of PCR results on retested 2017 PP outbreak samples), and 9.8 days vs. 26.6 days when using bacteriology.

The communication of the results to the MoPH for the national database was made within one day for partial results (RDTs and molecular biology) and 10 days for the final results (bacteriology).

Since the beginning of the 2019-2020 plague season, 27 notified plague cases have been received, with a confirmation rate of 78.6%.

Activity	Quantity (03/10/2018 – 30/09/2019)	Indicators (Achievement October 3 to September 30, 2019)
RDT test done	263 samples tested	100%
qPCR test done	288 qPCR reactions (234 samples tested once and 27 samples tested twice)	100%
cPCR	37 samples tested	100%
Culture (Bacteriology)	261 samples tested	100%
Differential diagnosis (classic bacteriology and Malditof)[&]	13 samples tested	100%
IgG Serology Rodent	855 samples tested (Investigation from Midongy, Tsiroanomandidy, and Anosizato-Ambanidia-Tsaramasay Mandoto – Betafo, Fianarantsoa, Toamasina) 198 (Investigation from Moramanga, notified cases from Faratsiho)	100%
IgG Serology Human	746 tests done (516 Direct ELISA, 230 ELISA inhibition)	100%
IgM Serology Human (investigation)		100%
Number of RDT and sampling kit distributed	755 RDTs distributed	For 20 Health District Services
Number of shipment from CSB to IPM Lab with USAID support	144 shipments	From 29 Health District Services to IPM lab
Recruitment of personnel	One senior lab technician (Oct 2018- June 2019), One lab technician (July- August 2019)	
Purchase order	46 229,06 €	
Analyses done at Clinical Biology Centre-IPM (for PP)		

Impact: Based on the various techniques that we are using at the CLP, an evaluation of their sensitivity and specificity will be performed to suggest the appropriate gold standard for plague confirmation. Related papers are being prepared.

Furthermore, as the culture of *Y. pestis* may take more than 10 days, we will attempt to identify conditions that reduce this period and improve the yield of bacteriological culture.

With the use of USAID funds linked to the 2017 epidemic, a mobile laboratory, or Laboratory on Wheels (LOW), adapted to the Madagascar road system has been purchased and prepared in Germany. The mobile laboratory reached Madagascar in June 2019 and its inauguration was held in September 2019 at the IPM. The mobile laboratory is equipped with a biosafety cabinet and can be equipped with diagnostic equipment, enabling molecular diagnosis in the

field to avoid the delay of several days for the biological samples to be transported to the capital city for analysis.

These activities will be continued for the next five years in the frame of the RISE project.

Subproject SP29: Plague: strengthening community health education and epidemiological surveillance

Key leaders: Dr Rindra Randremanana & Dr Chiarella Mattern

Background: Plague is endemic to Madagascar. Although certain plague foci are known in the country and people are aware of this disease, the risk of outbreak is still present. From August 1 to November 26 of 2017, a large scale pneumonic plague outbreak deeply affected the country. Several units of the IPM were mobilized during this period, especially the CLP of the MoPH, at the IPM Plague Unit, to ensure case registration and confirmation of laboratory tests, and the Epidemiology and Clinical Research Unit (EPI-RC), which supported the Plague Unit in epidemiological surveillance, outbreak investigation, data management and socio-anthropology research. To complete the data from Subproject SP25, the Social Sciences Team of the EPI-RC conducted a second survey to improve the surveillance and alerting of and response to plague endemics in Madagascar.

Study objective: To highlight the difficulties encountered by healthcare workers for the management of care for hospitalized patients (difficulties related to announcing the disease to the patients, management of the patients, structural difficulties or diagnosis, management of the corpse, and management of the victim's families), as well as the perception of the disease by the population and the impact of official and unofficial communication on public health measures.

Methodology: Eighteen healthcare professionals were interviewed between December 2018 and February 2019 in the second main hospital of the capital: Befelatanana hospital. All interviews were transcribed and translated and the data analyzed by theme. Between December 2018 and 2019, socio-anthropologists participated in the field investigation in Midongy and Mantasoa/Moramanga. In total, they conducted 26 interviews with recovered patients, victims' families, witnesses, the general population, and one focus group.

Results and discussion: The results of this survey showed that plague is a known disease, but that its characteristics remain unclear for most of the population, including its causes, its transmission pattern, and the distinction between pulmonary and bubonic plague. Plague was systematically associated with a lack of hygiene and poverty, which exacerbated the feeling of shame and stigmatization of sick people by those around them. The progressive awareness that anyone can be infected by the plague comes up against the social representations of the disease, leading to fear. This perception often leads to misunderstanding and refusal of the diagnosis by the patients, encouraging some of them to run away from hospitals. These shortcomings and the general lack of information from the authorities during the epidemic maintained a sense of panic and reinforced fear. They also promoted the emergence and circulation of rumors and conspiracy theories through the mass and social media. These rumors, as well as fear and misrepresentations of the disease, influenced the behaviors that were adopted throughout the epidemic (e.g., self-medication, caregivers' behaviors to prevent giving the disease to their family) and have had an impact on public health measures.

Impact: The results of these qualitative plague studies have helped to strengthen the message for plague awareness: between June and December 2019, we participated with other technical partners and the MoPH (lead by Dr. Sabine from the Direction de Promotion de la Santé) in the process of validating the communication strategy on the fight against plague and updating the message**.**