Zika virus induces cell death in human iPSC derived neuronal cells

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The major concern in the current Zika virus outbreak in South America is the association with fetal abnormalities and neurological complications. While more evidences of vertical virus transmission are reported, little is known about the target cells and mechanism of infection in the fetus. To investigate the potential target cells of the Zika virus in the central nervous system (CNS) we infected two types of neuronal lineage cells differentiated from human induced pluripotent stem cells (iPSC) with ZIKV strain MR766. Both cortical neurons and motor neurons were susceptible to Zika virus infection. Cortical neurons were more susceptible to ZIKV infection, resulting in full CPE at day 7 p.i. with an MOI as low as 0.00001, while for motor neurons no CPE was observed at the same MOI at day 12. The ZIKV RNA levels and the infectivity of viral progeny were quantified at different time points p.i. by qRT-PCR and end-point titration on Vero cells, respectively. Both types of neurons were able to produce infectious virus. The level of maturity of the cortical neurons cells did not influence ZIKV infection.

Next, the antiviral activity of inhibitors with different mechanisms of action was studied in the both types of neurons: T-705 (favipiravir), 7-deaza-2'-C-methyladenosine (7DMA) and ribavirin. Interestingly, only 7DMA was able to inhibit ZIKV-induce CPE and ZIKV replication in the neuronal cells, whereas all tested inhibitors exhibited an anti-ZIKV effect in Vero cells.

In conclusion, our results show that both cortical and motor neurons can be the target cells of ZIKV infection. However, the role of cortical and motor neuron infection in the development of fetal CNS abnormalities associated with ZIKV will require further investigation. Furthermore, we demonstrate that human iPSC derived neuronal cells can be a relevant *in vitro* tool to study ZIKV neurotropism and to test antiviral drugs.

ZIKA virus replication in human placenta

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Zika virus (ZIKV) is an emerging arbovirus of the *Flaviviridae* family transmitted to humans by mosquito bites and sexual route. ZIKV caused a major outbreak in French Polynesia in 2013/2014 and has since spread in 2015 to the Americas, notably in Brazil. Most cases of zika infection in otherwise healthy adults are asymptomatic or pauci-symptomatic, with fever, rash, arthralgia, and conjunctivitis. ZIKV infection has recently been associated with severe fetopathy, including microcephaly (Brasil et al., N Engl J Med. 2016, in press). Placental calcifications are observed in ZIKV-infected pregnant women and the virus is also present in the amniotic fluid and brain of fetuses with microcephaly (Calvet et al., Lancet Infect. Dis., 2016, in press; Mlakar et al., N Engl J Med. 2016, in press; Sarno et al., PLOS Negl Trop Dis, 2016). Together, these data strongly suggest that ZIKV has ability to cross the placental barrier and induce fetopathy.

Here, we have assessed the ability of ZIKV to infect human placental explants and human trophoblast cell lines, as compared to Chikungunya virus (CHIKV), another emerging arbovirus which is not able to infect the placenta and is exclusively transmitted vertically from viremic mother to their baby during parturition via placental breaches. We infected third trimester human placental explants with either ZIKV or CHIKV, and viral load in placental tissues and supernatants were determined daily from day 1 to day 4 post-infection. We found that ZIKV replicates in human placental explants, as shown by viral titers in placental tissues and supernatants, while, as expected, CHIKV does not. The identification of the cells targeted by ZIKV in human placental explants is currently investigated. In addition, a human trophoblast cell line was shown to be susceptible to ZIKV infection, but resistant to CHIKV infection. These preliminary data suggest a direct ZIKV tropism for human placenta. The relevance of these results will be assessed by detection of ZIKV in human placental biopsies of infected pregnant women. Studies in animal models susceptible to ZIKV will help to decipher how and when this virus crosses the placental barrier.

Deciphering the placental phase of ZIKV infection is required to understand its vertical transmission and develop potential preventive and therapeutic strategies against this emerging teratogenic arbovirus.

Zika Virus Infection with Prolonged Maternal Viremia and Fetal Brain Abnormalities – Virology findings

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The current outbreak of Zika virus (ZIKV) infection has been associated with an apparent increased risk of congenital microcephaly. We describe a case of a pregnant woman and her fetus infected with ZIKV during the 11th gestational week. ZIKV RNA was identified in maternal serum at 16 and 21 weeks of gestation. At 19 and 20 weeks of gestation, substantial brain abnormalities were detected on ultrasonography and magnetic resonance imaging (MRI) without the presence of microcephaly or intracranial calcifications. On postmortem analysis of the fetal brain, diffuse cerebral cortical thinning, high ZIKV RNA loads, and viral particles were detected. The highest viral loads were found in fetal brain, with substantial viral loads in the placenta, fetal membranes, and umbilical cord. Lower amounts of ZIKV RNA were found in fetal muscle, liver, lung, and spleen as well as the amniotic fluid at the time of termination. Maternal serum that was obtained on the day before termination was also positive for ZIKV RNA with a low viral count. No ZIKV RNA was detected in the serum, peripheral-blood mononuclear cells, saliva, or urine of the mother 11 days and 13 days after termination.

Infectious ZIKV was isolated from fetal brain in SK-N-SH (human neuroblast) and Vero-E6 (green monkey kidney cells). A complete ZIKV genome was sequenced from the isolate. Phylogenetic analysis indicated that the viral strain was a member of the Asian genotype and closely related to two ZIKV sequences obtained from Guatemalan patients who presented with mild illness. The virus strain had 23 to 51 nucleotide differences and 8 to 14 amino acid differences as compared with the ZIKV strains detected previously in the Americas (99.6 to 99.8% identities).

This study highlights the possible importance of ZIKV RNA testing of serum obtained from pregnant women beyond the first week after symptom onset. The isolation of ZIKV from fetal brain provides additional evidence for the association between congenital ZIKV infection and fetal brain damage and provides tools for further studies of the pathogenesis of ZIKV-induced microcephaly.

Phylogeography and molecular evolution of Zika Virus

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Zika virus has recently been associated with neuropathology. We analyzed viral genetic sequence datasets to reconstruct how Zika virus has and is spreading from Africa to Asia, the Pacific and the Americas. We identify key mutations in the polyprotein that occurred as Zika spread from the Pacific to the Americas. Using novel geographic visualization techniques we find that the Asian clade starts in Malaysia, but shares recent common ancestry with a lineage from West Africa. This result is distinct from that described by Faye et al., 2014 who link the Asian clade to Uganda. The result also differs from that of Lanciotti et al., 2016 who link the Asian clade to the common ancestor of an all-Africa clade. Our result does not designate a separate monophyletic all-African clade, but rather a set of African lineages from which the Asian-Pacific-Americas clade descends. Our tree also depicts a long branch connecting the Asian-Pacific-Americas clade to common ancestors shared with West African isolates, indicating the lack of sampling of the virus in the middle of the 20th century. More data will be required to clarify the phylogenetic history of the virus in the Eastern hemisphere and its potential threat to Africa, Asia, and Europe.

Point-of-care quantitative diagnostics of Dengue and Zika using magnetic nanoparticles

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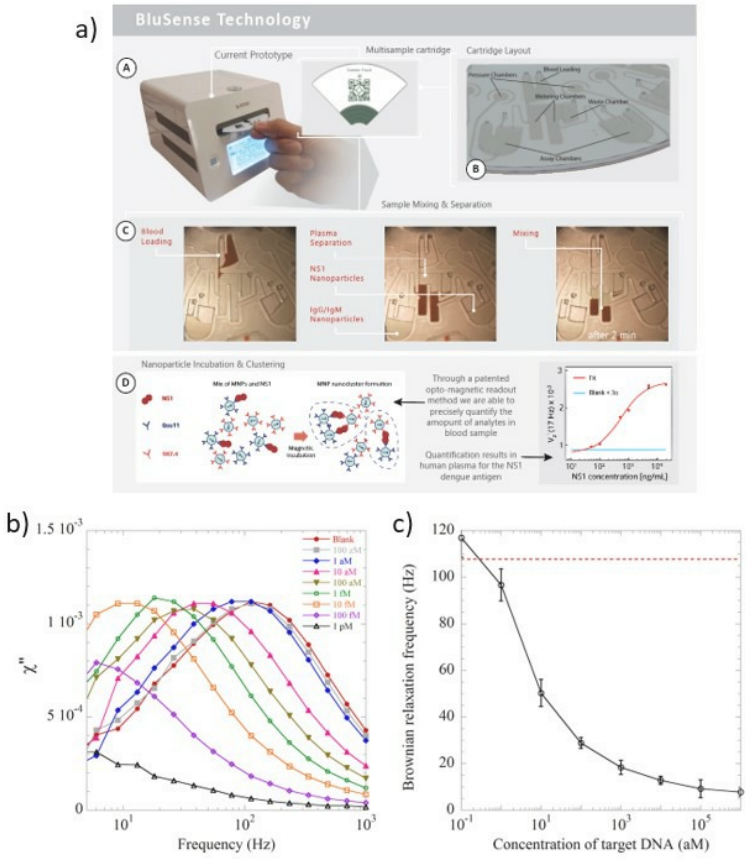
BluSense Diagnostics is developing an innovative blood test point of care technology that can impact mosquito-borne diseases diagnostics, such as Zika, Dengue and Chikungunya. BluSense technology is based on the use of plastic cartridges and on a compact, portable reader, roughly the size of an external Blu-Ray player. As illustrated in Figures 1, the test is performed simply by introducing a drop of blood in the cartridge, and by loading in the reader. For serological tests, the plasma is separated from red blood cells by centrifugal forces in the cartridge, and split in several chambers for combined NS1-IgG and IgM detection and mixed with magnetic nanoparticles (MNPs) coated with high-affinity monoclonal antibodies against NS1 or specific antigen for antibodies detection. The presence of the target triggers MNP agglutination and the formation of nanoclusters with rapid kinetics enhanced by external magnetic actuation. The amount and size of the nanoclusters correlate with the target concentration and can be quantified using the proprierty optomagnetic readout method [1]. In addition to serological tests, collaborators at Uppsala University have demonstrated as well an ultrasensitive molecular based assay for synthetic Zika DNA sequence using the same readout method. Target sequence, corresponding to a conserved region of the viral genome, is recognized, amplified and labelled in a one-step reaction in a sealed container by loop-mediated isothermal amplification (LAMP) generating biotinylated amplicons. Inclusion of streptavidin-coated 100 nm magnetic nanoparticles showing Brownian relaxation behaviour in the reaction mixture results in binding of amplicons and $Mg_2P_2O_7$ (by-product of LAMP reaction) to the nanoparticle surface. An increased amount of target results in a down-shift of the MNPs Brownian relaxation frequency measured with Dynmag instrument (see panel b). Within a total assay time of no more than 35 min a sensitivity of 1 aM is achieved in 20% serum, see panel c). It has been demonstrated that, using the same reagents, this assay can detect both DNA and RNA target sequences.

[1] P.Antunes et al. *Sci.Report,* 5,1646(2015)



Highly specific serodiagnosis of acute and past Zika virus infections by NS1-based ELISA

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Background: Currently, Zika virus (ZIKV) infections spread rapidly in Latin America. While being a rather mild disease in immunologically competent persons, evidence is found that ZIKV infection during pregnancy may cause microcephaly in fetuses or lead to Guillain-Barré syndrome (GBS). To evaluate this, reliable and rapid diagnostics are needed, but yet available serological tests are limited by high cross-reactivity.

Methods: Recombinant ZIKV non-structural protein 1 (NS1) was expressed in HEK293T cells, purified and used to develop an enzyme-linked immunosorbent assay (ELISA) for the determination of circulating anti-ZIKV IgM and IgG antibodies. ELISA sensitivity and specificity were evaluated with 29 sera from ZIKV infected patients and 799 healthy individuals, respectively. Samples from 128 patients with any of dengue, West Nile, Japanese encephalitis and chikungunya virus infections or Yellow fever vaccinated individuals were measured as a risk group of being infected with ZIKV.

Results: 28/29 ZIKV infected patients tested positive for anti-ZIKV NS1 IgM/IgG (96.6% combined sensitivity). Specificity based on the healthy individuals was 99.7%. Out of 128 sera from patients infected with dengue, West Nile, Japanese encephalitis and chikungunya virus or Yellow fever vaccinated individuals, 1 (0.8%) showed a positive reaction in IgG and IgM each.

Conclusions: Recombinant ZIKV NS1 provides a suitable molecular basis for the serological diagnosis of acute or past ZIKV infections. Providing a high sensitivity and specificity and a remarkably low cross-reactivity with other flaviviruses, our ELISA can serve as a specific diagnostic tool to elucidate the coherences among ZIKV infections, GBS and microcephaly.

Historical Perspectives on *Aedes Aegypti* Suppression and Eradication Efforts in the Americas

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In 2016, the government of Brazil launched the largest military campaign in its history to combat *Aedes aegypti,* the principal vector for Zika virus. Several hundred thousand uniformed soldiers and civilians have been enlisted to drastically reduce the vector density in an effort to stop transmission of the virus.

This is not the first military-style campaign against *Aedes aegypti* in the Americas. In the 1930s and 1940s, Brazil organized a similar campaign. Through aggressive larval source reduction programs, Brazil eradicated *Aedes aegypti* in the 1940s. In the late 1940s most other states in the Americas agreed to follow suit, in order to end the threat of urban yellow fever in the Americas. By 1964, most of the countries in South and Central America had eradicated the vector from their national territories.

The United States signed the 1947 agreement to eradicate *Aedes aegypti*, but did not pursue this goal until the period 1963-1967. In 1968, David Sencer, the director of the CDC, argued against the ongoing efforts to eradicate *Aedes aegypti* from the southern United States, and the U.S. program ground to a halt. Other states in the Americas likewise abandoned their eradication programs. Within a decade or so, *Aedes aegypti* had reestablished itself throughout its former range.

The arguments of the 1960s against *Aedes aegypti* eradication were based on considerations of yellow fever transmission. How may the calculus change when we consider dengue, chikungunya, and zika (as well as yellow fever)?

This presentation develops some historical perspectives on past efforts to eradicate or suppress *Aedes aegypti* in the Americas. It suggests that the older framework of intervention modalities—top down or bottom up—will need to be rethought in the light of Brazil's new military commitment, and that the efforts to suppress or eradicate *Aedes aegypti* are more urgent from a public health perspective and more attractive from an economic perspective than in the twentieth century.

Collaborative data sharing in outbreak situations

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Large collaborative projects offer significant rewards and challenges in equal parts. In the pursuit of success collaborators must navigate a myriad of legal, ethical, and communicative hurdles. While the internet is our greatest tool for communication it is also a source of concern, particularly surrounding issues such as data security and maintaining control over correct legal and ethical compliance. For the Milieu Interieur project (hosted by the Institute Pasteur, Paris, and lead by Matthew Albert and Lluis Quintana-Murci) we have adopted Synapse (https://www.synapse.org/) as our collaborative and public facing tool, allowing fine-grained control over user access and data-specific compliance. Synapse is an open source software platform that scientists use to carry out, track, and communicate their research in real time. The platform enables co-location of scientific content (data, code, results) and narrative descriptions (wiki) of that work. The aim of Synapse is to provide an environment for living research projects, i.e. projects whose data lives on to contribute to society beyond original research goals. Synapse has been used heavily in international collaborative projects like the Dream challenges (http://dreamchallenges.org/). We envision that Synapse will prove equally valuable in the very challenging situations of an outbreak, where coordination is key to acting rapidly.

Microcephaly and Zika infection: Preliminary Report of a Case-Control Study

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Background: The strongest hypothesis as to the cause of the recent microcephaly epidemic in Brazil is Zika virus (ZIKV) infection during pregnancy.

Methods: This is a preliminary report of a .case-control study conducted by MERG, the Microcephaly Epidemic Research Group, to investigate the association between microcephaly and Zika infection during pregnancy. A total of 23 cases and 45 controls were recruited prospectively between 15 /01/2016 and 01/04/2016. Cases were neonates, with microcephaly (head circumference below 3rd percentile for gestational age and sex) born in selected hospitals in the city of Recife, Pernambuco, Brazil; controls were neonates born in the same hospital matched by expected date of delivery and area of residence. Serum of cases and controls and Cerebral Spinal Fluid (CSF) of cases were tested for Anti-Zika-IgM and quantitative reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assays. Neonates were considered to have serum-laboratory-confirmed Zika if IgM or RT-PCR were positive in serum; cases were also classified according to whether they tested positive in either serum or CSF.

Results will be presented .

Introduction and Emergence of Zika virus in French Guiana

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Since the identification of Zika virus (ZIKV), in Brazil in May 2015, the virus has spread throughout the Americas. On December 17th 2015, the Institute Pasteur in French Guiana confirmed that 10 individuals returning from a mission in a neighbouring affected country were carrying ZIKV. On December 18th, the first locally acquired case of ZIKV disease was confirmed with patient of the coastal and urbanized area.

Health authorities and workers involved in epidemiological surveillance, health care and vector control focused on educating clinicians and general population, improving diagnosis tools, monitoring systems and vector control activities to implement rapid measures to limit the spread of the virus.

The spatiotemporal evolution of the confirmed cases during the four weeks following the first cases highlighted a rapid spread of the disease. On January 22nd, 2016, the local health authorities launched an official epidemic alert throughout the coastal urbanized area regrouping 90% of population. From the first detection of ZIKV to the 1st of April 2016, 355 cases were confirmed by RT-PCR and 3 620 clinically suspect cases were estimated by the Institute for Public Health Surveillance. A total of 165 pregnant women and 2 Guillain-Barré syndromes were identified as positive cases.

The complete genome of the ZIKV was obtained for three patients and NS5 protein coding region sequences for others patients. Sequences and phylogenetic analysis showed that strain from both countries cluster together and belonged to the Asian genotype and appear to be most closely related to the strain that was circulating in French Polynesia in 2013, with which they share more than 99.7% and 99.9% of nucleotide and amino acid identity, respectively.

Considering various existing potential obstetric risks and the important birth rate in the country, the outbreak represents a particular threat in French Guiana. While the greatest majority of the cases might cause mild illness, the potential impact of the outbreak is postulated to be one of the most important challenges for health authorities in order to anticipate the mobilisation of extra resources and to prepare for adequate care capacity to monitor pregnant women and to manage the most severe cases.

The viral polymerase inhibitor 7-deaza-2'-*C*-methyladenosine inhibits *in vitro* Zika virus replication and delays disease progression in a robust mouse infection model

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Given the potential link with microcephaly, Zika has been declared a public health emergency of national importance in Brazil. There is neither a vaccine nor a specific antiviral therapy for the prevention or treatment of infections by ZIKV. The increasing incidence of Zika fever stresses the need for both preventive and therapeutic measures. We here report on the establishment of (i) a panel of assays that allow to identify inhibitors of ZIKV replication (including by high throughput screening) as well as (ii) a robust animal model of ZIKV infection. Infection of AG129 (IFN-α/β and IFN-γ receptor knock-out) mice with ZIKV resulted in acute neutrophilic encephalitis with viral antigens accumulating in neurons of the brain and spinal cord. High levels of viral RNA were also detected in the spleen, liver, kidney and interestingly in testes. Levels of IFN-γ and IL-18 were systematically increased in serum of ZIKV-infected mice. The viral polymerase inhibitor 7-deaza-2'-*C*-methyladenosine (7DMA) was identified as an inhibitor of *in vitro* ZIKV replication. Moreover, 7DMA reduced viremia and delayed the time to disease progression in virus-infected mice which also validates this animal model to assess the in vivo efficacy of novel ZIKV inhibitors. This model may also be useful to assess efficacy of vaccines against ZIKV; indeed AG129 mice have been intensively used in dengue vaccine research.

Experimental studies of susceptibility of Italian *Aedes albopictus* to Zika Virus

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Zika virus (ZIKV) is a single-stranded RNA virus (Family *Flaviridae*, genus *Flavivirus*) transmitted to humans mainly through the bite of infected *Aedes* mosquito, primarily *Aedes aegypti*.

The purpose of this study was to evaluate the vector competence of a laboratory colony of Italian *Ae. albopictus,* using as positive control a laboratory colony of *Ae. Aegypti*..

Experimental infection was performed by a membrane feeding apparatus and an infectious blood meal at a final concentration of 10^6 PFU/ml.

Ten-day old females were fed and monitored, after the infectious blood meal, for 24 days (T=26±1°C, 70% RH, light/dark of 14/10 h) to determine the length of viral extrinsic incubation period. ZIKV titre of infected mosquitoes was evaluated by quantitative Real Time PCR. Infection, Dissemination and Transmission Rates (IR, DR, TR) were assessed by detection of the virus in abdomen, legs plus wings and saliva, respectively, of mosquitoes collected at 3, 4, 7, 11, 14 18, 21 and 24 days post infection (pi).

Preliminary results indicated that *Ae. albopictus* were able to become infected and the virus to disseminate. Starting from day 11 pi, ZIKV was present in legs and wings in 10% of tested mosquitoes, and the virus was also detected in the saliva in 50% (TR) of the infected mosquitoes. However the mean viral titres in legs, wings and saliva of the infected *Ae. albopictus* were lower than those detected in infected *Ae. aegypti*. Furthermore, in *Ae. aegypti* the presence of ZIKV in legs, wings and saliva was detected starting from day 4 pi, with values of DR and TR of 17% increasing up to 100% in following collection times.

Although the specimen analyses are still in progress, our preliminary results have shown the susceptibility of the Italian *Ae. albopictus* colony for ZIKV but with lower vector competence than the main vector *Ae. aegypti*.

Aedes aegypti trapping systems coupled with sugar baits for early detection of arboviruses: demonstration with Chikungunya virus, application to Zika virus

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Background:

In French Guiana and neighboring countries, dengue is endemo-epidemic since many years, chikungunya hit the region in November 2013 and is now well installed and zika has just emerged causing an unprecedented outbreak. These diseases are mainly transmitted by the mosquito *Aedes aegypti*. Entomological surveillance is a prerequisite to identify hot spots of transmission and to thereby guide vector control interventions. This surveillance usually relies on the processing of large amount of living mosquitoes collected on the field with appropriate trapping methods. This procedure is impeded by manpower constraints that are rarely compatible with logistical capabilities of control operators and implies the processing of fresh mosquitoes in order to avoid virus degradation. Recent work suggested that mosquito sugar feeding could be exploited to capture and preserve virus RNA expelled in their saliva.

Methods:

We modified traps usually used to collect *Ae. aegypti* in order to preserve arboviruses RNA passively expelled on the field by infectious mosquitoes. The devices were tested in the urban area of Cayenne, during the 2014-2015s' chikungunya epidemic are have been deployed around the first autochthonous zika cases recently reported by local sanitary authorities.

Results:

We showed that Chikungunya virus could be detected by real time RT-PCR on nucleic acid preservation cards impregnated with honey and integrated in ovitraps. The cards installed from the traps set around zika cases are under investigation.

Conclusions:

Early detection of Chikungunya virus expelled by mosquitoes on honey impregnated cards during their sugar meal presents an interesting alternative, highlighting the potential of a simple, cheap and energy-free ovitrap for monitoring arboviruses circulation in the field, before or in addition to epidemiological surveillance, or in locations where no epidemiological data are available. Improvement of the method will be discussed.

A novel contamination device that targets multiple life-stages of *Aedes* vectors

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The increasing global threat of Zika virus demands new and effective vector control methods. *Aedes* mosquitoes are difficult to control as they divide their eggs over various small and hard to find breeding sites, and have become resistant to chemical insecticides. We here present the In2Care® Mosquito Trap, a novel tool to combat multiple life-stages of *Aedes* mosquitoes.

The device delivers adulticidal, larvicidal and auto-dissemination impacts by deploying a unique combination of pyriproxyfen larvicide and *Beauveria bassiana* fungus spores. In2Care® Traps attract gravid female mosquitoes and contaminate them with high doses of biocides through a special electrostatic netting strip. The product exploits the concept of 'auto-dissemination', as pyriproxyfen gets spread to multiple breeding sites by contaminated skip-ovipositing *Aedes* females, resulting in an effective kill of mosquito larvae in the trap vicinity.

Semi-field and field studies in the USA, Trinidad and Grand Cayman islands have delivered evidence of trap impacts on wild-type mosquitoes under field conditions. Results showed high attraction to breeding *Aedes aegypti* and *Ae. albopictus*, high egg dump capacity; 100% larval mortality inside the traps, and significant fungus-induced adult mortality impacts. On Grand Cayman, traps were shown to reduce the breeding success (adult emergence rates) in sites near the traps with more than 45%.

In2Care® Mosquito Traps have particular value for vector control in targeted problem areas where Zika virus transmission risks are high and where insecticide resistance or cryptic breeding is causing problems. We will present the current product registration status and user cases from governments, pest control operators and consumers to illustrate operational features and trap deployment options.

Response to the first zika outbreak in Martinique (French West Indies)

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In Martinique, dengue, chikungunya and zika viruses are transmitted by Aedes aegypti. The main breeding sites of this mosquito are generated by human practices. The last dengue outbreak occurred in 2013 and the first chikungunya outbreak in 2014.
The first autochthonous case of zika was confirmed on December 18th 2015. According the usual protocol established for dengue and chikungunya control, the Mosquito Control Center team organized a “blitz”; it is an operational method to control an emerging arbovirus focus. The “blitz” consists, with collaboration of the municipality staff, in (i) a dissemination of prevention messages in the area around the clinical case, (ii) a systematic removal and suppression of larval breeding sites, (iii) a systematic indoor spraying and (iv)a spatial outdoor spraying.
The main goal of this approach is to stop the virus spread. However it did not stop the spread of zika in the whole island. Possible explanations could be the high level of resistance of mosquitoes to deltamethrin (the only adulticide used in France), the difficult access to houses (closed, abandoned…), the presence of remaining breeding sites.
So, the zika outbreak was officially declared by the Health Authorities in January 20th 2016. The latest data raised on week 15: 15 440 clinical cases, 106 pregnant women with zika, 8 Guillain-Barre Syndrome cases with zika.
According to the dengue and chikungunya outbreak experiences and because of entomological parameters (type of breeding sites, resistance to insecticide...), we avoided the use of insecticide as the front line of defense against zika. Then our working lines were:
- participation of decision makers,
- information and communication,
- breeding sites suppression,
- entomological surveillance in “sensitives” sites (hospitals, schools, hostels…),
- individual protection (use of repellent),
- environmental management,
- larvicide (BTI) application in some breeding sites,
- social mobilization (“Zika global mobilization week”, 20-27 feb.2016).

The operational considerations and difficulties of our experience in Martinique will be presented, discussed and shared.

Zika virus infection causes microcephaly-like effects in iPSC-derived human brain organoids

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Primary microcephaly is a rare developmental genetic disorder, known to be caused by mutations in centrosomal genes. Thus far, it has only affected around 200 patients worldwide. However, the recent Zika virus (ZIKV) outbreak in Brazil saw a 25-fold increase in microcephaly-associated cases, leading to high emotional and economic burdens for patients and their families (Brasil et al., 2016; Tang et al., 2016; Ventura et al., 2016). Thus, insights into the tropism and mode of action of ZIKV in the developing human brain are urgently required. Here, we demonstrate that the French Polynesia ZIKV, a strain associated with microcephaly and genetically almost identical to the Brazilian isolate, indeed efficiently infects and is highly productive in human brain organoids derived from induced pluripotent stem cells (iPSC). ZIKV infection induces massive cell death disrupting neuronal cells in the cortical region. Through this mode of action, ZIKV-infection may reduce cortical volume, an observation that is consistently found in microcephaly patients (Alcantara and O'Driscoll, 2014; Alkuraya et al., 2011; Guernsey et al., 2010)·(Bond et al., 2005). Thus our study provides a mechanistic explanation for the reduced neurogenesis and brain size in ZIKV-infected newborn babies.

In the footsteps of Zika…following the path of Dengue and Chikungunya?

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While until recently the small and/or isolated Zika outbreaks in Pacific islands and Eastern Asia have been overlooked, the large scale outbreak that started in Brazil in 2015 and the increased of cases of microcephaly cases in the same place and time hit the headlines in the medias. Considered until now as harmless, Zika has created a worldwide crisis. That was the fourth time only that the World Health Organization has identified the disease caused by that virus as a public health emergency of international concern in accordance with the International Health Regulations (2005) after H1N1 Influenza (2009), Poliomyelitis (2014) and Ebola (2014). Based on the rapid geographical spread of Dengue and Chikungunya that use the same vector Aedes genus mosquitoes, Zika's spread live maybe in way more than we think and the landscape for vector-born diseases is likely to be redrawn. Although mapping the global distribution of those vectors (temperature, rainfall, rural-to-urban habitat) and ecosystemic determinants of transmission of pathogens & viral replication remain essential to public health planning, an interdisciplinary approach will be critical for better understanding the Zika phenomenon. Climate change is probably as great an influence as increased global movement of people for the spread of Zika, which is, transmissible from mother to child, sexual contact blood transfusion unlikely to Chikungunya and Dengue. This is likely to become an unequal crisis between countries. Low-income countries will probably be disproportional more impacted than others in terms of vector control and exposure. However, to what stage wealth and technology will protect developed countries from Zika?

Variability of the basic reproductive number (R_0) for Zika epidemics in the Pacific islands

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Before the widely publicized outbreak that reached the Americas in 2015, Zika has been circulating in Asia and the Pacific where several outbreaks have been reported in Micronesia, French Polynesia and New Caledonia between 2007 and 2015. These past outbreaks can be highly informative on the key parameters driving virus transmission, such as the basic reproduction number (R_0), and on their variation in different settings. Studying these outbreaks is thus key to our understanding of the current Zika epidemics.

In order to assess the variability of R_0 across populations of different sizes and locations, we are comparing estimates for several outbreaks in Pacific islands. Variation in the model formulation is also analyzed, as parameters related to the mosquito population have noticeable impact on the model‘s outcomes. For each setting, we consider two compartmental models using different vector representations. Simulation of the epidemic dynamics includes stochasticity and the estimation of the parameter set best fitting the incidence data is performed using pMCMC particle filters.

Comparing results across settings and models, R_0 for the Pacific Zika epidemics is estimated between 1.5 and 4 (with the Next Generation Matrix calculation). The largest values are obtained on the smallest population size. Nevertheless insights on spatial heterogeneity and cross-reaction between flaviviruses are needed to better understand the Zika virus transmission drivers.

Clinical Characterisation of Zika Virus Infection in the Context of Co-circulating Arboviruses in Latin America - the WHO-IDAMS-ISARIC Protocol

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The WHO-IDAMS-ISARIC protocol offers a master clinical characterisation protocol that includes a standardised natural history data collection tool (case report form) with the option for biological sampling. This international collaboration joins the expertise of WHO and ISARIC with regard to protocols for severe acute respiratory infections and viral haemorrhagic fevers, with the ongoing clinical characterisation studies being undertaken in Latin America by the International Research Consortium on Dengue Risk Assessment, Management, and Surveillance (IDAMS), which have been adapted to include syndromes caused by Zika and Chikungunya.

The primary objectives of this standardised protocol are to gather data to:

1. Describe the clinical manifestations of Zika virus infection across a broad range of age groups and countries, taking into account a number of possible risk factors or effect modifiers potentially associated with certain clinical phenotypes.
2. Identify clinical and/or simple laboratory parameters distinguishing between Zika, Dengue, and Chikungunya infection at initial presentation, thus developing a robust case definition for Zika in the context of co-circulating viruses.
3. Identify clinical, laboratory, and virological parameters among patients infected with Zika, that are associated with poor prognosis or complications.

This protocol is aimed to be used as a prospective multi-centre observational study enrolling both adults and children with an acute febrile/ rash illness consistent with Zika, Dengue, or Chikungunya, at the time of presentation to a healthcare facility. Detailed clinical history and examination findings and laboratory results will be recorded in a standardised case report form. Patients will be followed up prospectively during the acute illness and contacted repeatedly over the following months in order to capture the full spectrum of disease.

A biosilica-based detection device for (re-) emerging arboviral infectious diseases

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Biosilica is a versatile material that can be used for a wide range of applications. It is produced by many marine organisms, but not only. One of the properties of biosilica is its ability to support biological functions. The 2016 iGEM Team of Institut Pasteur has set as aims to use biosilica in order to design and build a novel detection system for arboviral emerging diseases. The purpose is for the production of a tool or device that can be used in the field by professionals in assays that identify the signature of the organism. The team will consider all impacts of such a project, including ethical, biosafety, economical and human practices in areas where the tool will be used. We present here the general idea of the project that will be performed over the summer by a group of students with diverse backgrounds, ranging from law and design, to biology and chemistry.

Real time RT-PCR for detection of ZIKA virus

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Zika virus is an enveloped, single stranded (+) RNA virus of the family *Flaviridae*. Zika infection was associated with only mild illness prior to the large French Polynesian outbreak in 2013 and 2014, when severe neurological complications were reported. In 2015, the Zika fever spread to Brazil and more than 20 other countries in the South and Central America. The clinical presentation of Zika fever is nonspecific and can be misdiagnosed as other infectious diseases, and typically, 80%of Zika infections are asymptomatic. Because Zika, Dengue and chikungunya virus are endemic in the same geographic regions and cause similar symptoms, the identification of the etiological agents is only possible in laboratory testing.

Real-time RT-PCR (rRT-PCR) is an appealing option as rapid sensitive and specific method for detection of ZIKV in the early stage of infection. That is the reason why, an RT-PCR assay to detect specifically Zika virus was developped in our lab. Sequences retrieved from the Genbank database were used to designed primers and probe. We designed primers and probe specifics to NS5 and NS1 proteins, and also primers targeted envelope protein and capsid. Finally after in vitro screening specific primers and probe in the NS5 protein region were selected. Then, we optimized a rapid RT-PCR in one step (1 hour) using a reverse –transcriptase (RT) reaction to convert RNA into complementary DNA (cDna) in one step before PCR for the amplification of specific target. The assay also includes a G6PDH detection as control .We checked the specificity and our test is specific of ZIKA virus (Uganda and French Polynesia strains). Moreover no cross reaction was detected with CHKV , DENV serotypes, West Nile virus, and Yellow fever virus. The sensitivity is below 1pg of genomic RNA.

In conclusion, the assay is rapid sensitive and specific to detect ZIKV in cell culture but needs to be validated in serum and clinical samples for diagnosis using.

Evaluating viral interference between Influenza virus and Infectious bronchitis disease virus using real-time reverse transcription–polymerase chain reaction in chicken eggs

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Simultaneous and sequential allantoic cavity inoculations of Specific-pathogen-free (SPF) chicken eggs with Influenza virus (AIV) and Infectious Bronchitis disease virus (IBV) demonstrated that the interaction of AIV and IBV during co-infection was variable. Our research revisited the replication interference potential of AIV and IBV using real-time reverse transcription–polymerase chain reaction (real-time RT-PCR) for AIV and IBV to specifically detect the viral genomes in mixed infections. Data from this study showed that when different times of AIV (A/CK/TUN/145/12) and IBV (H120) inoculations into embryonating chicken eggs (ECE), interference with the growth of AIV or IBV occurred. When equal amount of the two viruses were sequentially employed, the degree of interference was dependent upon the time of super infection. The second virus inoculated has a negative impact on the growth of the first virus inoculated if they are inoculated sequentially.

Development of Zika virus reverse genetics, vaccine candidates and antibodies

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Zika virus (ZIKV) is a mosquito-borne flavivirus that is responsible of ongoing outbreaks in Americas and is associated with development of microcephaly in neonates and Guillain-Barre syndrome. The links with severe health concerns have raised the need for development of vaccines against ZIKV. The understanding of ZIKV lifecycle is at an early stage and is hampered by lack of molecular biology tools. This research was aimed to generate reverse genetics system for ZIKV including infectious ZIKV clone, to design and develop a set of ZIKV vaccine candidates and to generate antibodies to study ZIKV biology.

The infectious clone design of ZIKV is based sequences of clinical isolates from Brazil. Currently we are assembling it from synthetic DNA fragments. Additionally, recombinant ZIKV genomes bearing different markers will be developed.

For developing vaccine candidates of ZIKV, different approaches were used. As one approach, the vaccine strain 17D of Yellow fever virus (YF) was used for generation of chimeric viruses. The vaccine strain 17D of YF was used as a vector, where the preM-E region was swapped with the respective region of ZIKV virus. In another approach, alphavirus vectors (another class of arboviruses) were used. The alphavirus genome encodes two polyproteins, from which one is expressing viral non-structural proteins (replicase), meanwhile structural proteins are produced under the sub-genomic promoter. In the late stage of alphavirus infection the expression of structural proteins is abundant and therefore it is proposed to potentially elicit high immunogenicity. For the generation of alphavirus based vaccine candidates the structural proteins were replaced with structural proteins (C-preM-E) of ZIKV.

The second aim of the study was to generate antibodies against proteins encoded by ZIKV. In addition to antibodies against immunodominant E protein polyclonal antibodies will be raised against polymerase domain of non-structural protein 5 (NS5), helicase domain of non-structural protein 3 (NS3) and mature capsid protein of ZIKV. These proteins have been expressed in *E. coli*, purified as soluble recombinant proteins and used to immunise rabbits. E-glycoprotein will be expressed in mammalian cells and used to immunize rabbits and mice with aim to obtain panel of monoclonal antibodies.

ZIKAVax project: the exosome technology as promising platform for the development of a candidate vaccine against Zika virus

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Mosquito-borne Zika virus (ZIKV) has recently gained a medical importance following the large-scale epidemics in the South Pacific especially in French Polynesia in 2013. The rapid expansion of ZIKV in the South and Central America has been recently documented emphasizing the remarkable capacity of ZIKV to spread to non-endemic regions. An increase in congenital microcephaly cases has been recently associated to ZIKV in Brazil evoking a potential risk of prenatal transmission of the pathogen. Neurological complications such as Guillain-Barré syndrome were also documented during the major outbreak of ZIKV in South Pacific. No strategies for disease control nor vaccines against ZIKV are available to date.

The severity of ZIKV-associated disease in humans warrants the need for the development of candidate vaccines against ZIKV. The research laboratory on infectious tropical diseases PIMIT in La Reunion island and the biotechnology company Ciloa in Montpellier have decided to join their efforts on countering the potential public health threat caused by mosquito-borne ZIKV. Ciloa has created a new and unique technology allowing the targeting of any type of membrane proteins embedded in the exosome membrane. This technology represents a new solution for safe and affordable vaccines starting with only the coding sequences of viral envelope antigens and without using neither infectious material and nor artificial adjuvants. Fully native viral membrane proteins on exosomes mimicking perfectly the original proteins on native enveloped viruses have been successfully produced.

The purpose of the ZIKAVax project is to evaluate the capacity of a series of exosomes harboring different ZIKV antigens (ZIKV-exosomes) at eliciting protective humoral immune response in immunized small animals. ZIKAVax is organized in two steps using the exosome platform as a “magic bullet”: (1) design, development, and antigenic validation of series of ZIKV-exosomes and (2) judging the efficacy of ZIKV-exosomes at inducing protective humoral response in immunized animals. As first proofs of concept of exosome vaccine potential against ZIKV, it will be important to determine whether ZIKV-exosomes are particularly efficient at boosting antigen-specific humoral immunity in animals primed with a naked DNA or viral vector expressing viral antigens.

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Development of rapid and sensitive ELISA kits for Zika Antibodies (Envelop, NS1, prM, and Capsid) in animals and humans.

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Zika virus is an emerging mosquito-borne virus that was first identified in Uganda in 1947 in rhesus monkeys. Since the 1950s, it has been known to occur within a narrow equatorial belt from Africa to Asia. There is preliminary evidence that Zika is a cause of microcephaly in babies and Guillain-Barré syndrome in adults. There has been a large increase in the number of microcephaly and confirmed Zika virus infections. Zika virus (ZIKV), a member of the virus family Flaviviridae, is transmitted by daytime-active Aedes mosquitoes, such as A. aegypti. Zika virus is related to the dengue, yellow fever, Japanese encephalitis, and West Nile viruses. Effective vaccines for yellow fever virus, Japanese encephalitis, and tick-borne encephalitis have been develop but there are no vaccines for Zika virus. Zika virus codes for a polyprotein that is subsequently cleaved into capsid (C), precursor membrane (prM), envelope (E), and non-structural proteins (NS1-5). Like other flaviviruses, both structural and non-structural protein antibodies are detected during Zika virus infection. Zika virus (inactivated) or recombinant subunit vaccines using Zika proteins are being explored. Therefore, there is an urgent need to develop rapid and sensitive ELISA kits to detect and measure antibodies to various Zika proteins in animals (mouse, rabbit, and monkey) and humans. Such kits will be very useful to determine the efficacy of any potential Zika vaccines. ADI has cloned, expressed and purified several Zika proteins (Env, prM, Caspid, and NS1) in multiple hosts ((E. coli, HEK cells, and sf9 cells) and made antibodies. ADI has developed very sensitive antibody (IgA, IgG, and IgM) ELISA kits for mouse, monkey, and human samples. The ELISA kits are particularly designed for field application and require minimal instrumentation (room temp incubation, assay time 105 min, visual color development). Additional ELISA kits are being developed to discriminate between the Zika antibodies from that of related flaviviruses.

Zika virus is a candidate for application of nanofibre based system for noninvasive sublingual immunisation: new technology platform for printed vaccines

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The Zika virus is spreading rapidly mainly within South America and Mexico. There is a real expectation that this infection could be further transmitted by infected persons to North America, Europe and northern parts of Asia. Transmission by sexual intercourse has been confirmed and this therefore could represent a significant route for spreading infection to the northern hemisphere, where transmission of Zika virus is improbable via the bite of Zika virus infected mosquitos. Preventive noninvasive vaccination with a readily scalable vaccine is likely to be the only effective method to protect humans, especially women, against the serious consequences of Zika virus infection.

Sublingual noninvasive vaccination is a valid approach to Zika vaccination owing to its ability to induce both a mucosal (sexual transmission) and systemic response (mosquito´s bite, prevention of transplacental infection of foetus).

An optimized system based on biocompatible nanofiber mucoadhesive films has been developed and tested on pig model (PCT). The system is now ready for application for development of noninvasive sublingual vaccines including Zika virus vaccine. Noninvasiveness of vaccination in conjunction with the relative ease of vaccine production (technology of printed vaccines) and application comfort for vaccinees are the factors that support introduction of this technology. Noninvasiveness of vaccination is the advantage of the technology which can significantly shorten approval and registration process for new vaccines like that needed to suppress the spread of zika infection.

Transient Hearing Loss in Adults Associated with Zika Virus Infection

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On May 2015 an outbreak of Zika virus (ZIKV) was identified in Brazil. Zika virus infection has been linked to neurological disorders like microcephaly and Guillain-Barré syndrome in Brazil and in other countries. Despite that, hearing impairment and pray-motor skills were not described. Here we report three cases admitted in the Emergency Unit with hearing loss, tinnitus and dizziness. All patients reported acute symptoms of itching exanthema, arthralgia and headache. Audiometry exams were performed before, during and after otologic symptoms in two patients and revealed temporary bilateral sensorineural hearing loss. A positive serologic test confirmed ZIKV infection in all patients. Our finding suggests a possibly association between Zika virus infection and acute hearing loss, which may persist for up to four weeks.

Evaluation of immune response in human Zika virus infection

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Zika virus (ZIKV) infection in Brazil, was first described in 2015 and it overlapped with a significant increase in number of cases of Guillain-Barre syndrome in adults and also in neurological malformation in infants, including microcephaly. The role of the immune response in protection and/or development of severe cases of infectionremain to be addressed. This proposal aims to evaluate both the innate and acquired immune response in patients with different clinical forms of the infection. Especially seeks to identify specific immune signatures to different clinical forms of the disease and, in addition, assess whether there are cross-reactivities to virus chikungunya and dengue (DENV). To this, a consortium gatheringclinical investigators (Department of Bahia State Health), virologists (Federal University of Bahia), immunologists (Fiocruz, Bahia) and molecular biologists and immunologists (Département d'Immunologie Hôpitaux Universitaires Pitie-Salpetriere, Université Pierre et Marie Curie - France) is being formed. The lymphocyte responses to ZIKV peptides and recombinant proteins as well as the innate immune cell responsesto the virus will be evaluated using different methodologies, including mass cytometry (CyTOF) that simultaneously analyzes up to 30 parameters in a single tube. Antibodies from ZIKV-infected individuals will be evaluated in relation to antigenic specificity, neutralizing capacity andcross-reactivity with DENV-4 and chikungunya virus antigens. The results of this project would contribute to the understanding of pathogenesis of the disease, in addition to the characterization of dominant immune response that will be important for the development of diagnostic tests, vaccines and identification of potential drug targets.

Key words: Zika virus, microcephaly, Guillan-Barré, immune response, recombinant proteins, mass cytometry

Biology of Zika virus infection in human skin cells

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Zika virus (ZIKV) is an emerging arbovirus of the *Flaviviridae* family that includes Dengue, West Nile, Yellow Fever and Japanese encephalitis viruses, causing **a** mosquito-borne disease transmitted by the *Aedes* genus, with recent outbreaks in the South Pacific. Here, we determine the importance of the human skin in the entry of ZIKV and its contribution to the induction of anti-viral immune responses. We show that human dermal fibroblasts, epidermal keratinocytes and immature dendritic cells are permissive to the most recent ZIKV isolate, responsible for the epidemic in French Polynesia. Several entry and/or adhesion factors, among which DC-SIGN, AXL, TYRO3, and to a lesser extent, TIM-1, permitted ZIKV entry with a major role for the TAM receptor AXL. ZIKV permissiveness of human skin fibroblasts was confirmed by the use of a neutralizing Ab and specific RNA silencing. ZIKV induced the transcription of TLR-3, RIG-I and MDA5, as well as several interferon-stimulated genes, including OAS2, ISG15 and MX1, characterized by a strongly enhanced interferon-b gene expression. ZIKV was found to be sensitive to the antiviral effect of both type I and type II interferons. Finally, infection of skin fibroblasts resulted in the formation of autophagosomes whose presence was associated with enhanced viral replication, as shown by the use of Torin 1, a chemical inducer of autophagy or the specific autophagy inhibitor 3-Methyladenine. The results presented herein permit to gain better insight in the biology of ZIKV and to devise strategies aiming to interfere with the pathology caused by this emerging Flavivirus.

Zika virus: the African and the Asian-Pacific-American lineages

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Zika virus circulates in Africa in lineages that have sequence and host diversity (arthropods, human and non-human primates) but does not cause severe disease. In contrast, Zika virus in the Pacific, South America, and the Caribbean has recently been associated with severe neuropathology. Viral evolution may be one factor contributing to an apparent change in Zika disease. To address this possibility, we have employed computational tools to compare the phylogeny, geography, immunology, and structure of Zika virus isolates from Africa, Asia, and across the Pacific to the Americas. We identify and evaluate a set of mutations in human isolates of Zika virus the Asian-Pacific-Americas lineage that distinguish that lineage from viruses circulating in Africa. These findings support the working hypothesis that mutations acquired by Zika virus in the Asian-Pacific-Americas lineage contribute to changes in neuropathology in fetal and adult human hosts. These results can accelerate experiments required for confirmation of the link between Zika infection and neuropathology.

Combination of the sterile insect technique (SIT) with Wolbachia-based approaches for a safe and sustainable solution to control zika vectors

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Vector-borne diseases are becoming important public health problems and high economic burdens globally. According to the World Health Organization (WHO) more than 2,500 million people in over 100 countries are at risk of contracting dengue, chikungunya while Zika virus disease outbreaks have recently taken place in several parts of the world with possible associations to microcephaly and neurological disorders. In the absence of effective vaccines and drugs, the population control of vector Aedes mosquitoes remains the most promising way for disease control.

In response to Member States requests, the Joint FAO/IAEA Insect Pest Control Laboratory has developed the mosquito sterile insect technique (SIT) package, as a component of integrated vector management approaches, against both Aedes aegypti and Aedes albopictus. Mass rearing protocols, tools and equipment are available, sex separation methods or methods to overcome problems associated with the presence of females have been developed, irradiation protocols to sterilize males and females have been established and methods for handling, transport and release have been developed and are continuously refined, while robust pre-release and post-release monitoring methods are also established. During the last 10 years, our R&D work has resulted in a robust SIT package against Ae. aegypti and Ae. albopictus which is absolutely safe for humans and safe for the environment; and therefore a responsible and sustainable approach to control Aedes mosquito populations. We have combined the SIT with Wolbachia-based approaches thus eliminating the risk of releasing fertile and potentially disease transmitting females in the wild, while it also eliminates the risk of or purposeful establishment in the pest population.

In addition, through the technical cooperation programme of the IAEA, the mosquito SIT package is being transferred in response to FAO and IAEA Member State requests in South and South East Asia, the Indian Ocean, Latin America and the Caribbean and recently also Europe. Through these on-site technical cooperation projects, small pilot trials have been performed providing very encouraging results about the potential of SIT-based approaches to control Aedes mosquito populations, while more trials are in the pipeline. Last, but not least, there are neither intellectual property rights issues, nor the chance for resistance for SIT-based approaches because irradiation-induced sterility is a random process

Epidemiologic Surveillance and Research Activities to Address Zika Virus and Pregnancy: An Update from the U.S. Centers for Disease Control and Prevention

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The emergence of Zika virus in the Americas with its untoward impact on the developing fetus is resulting in a rapid response by the scientific and public health communities. The U.S. Centers for Disease Control and Prevention (CDC) responded by activating its Emergency Operations Center on January 22, 2016. Given the paucity of data specific to how the virus impacts the fetus, CDC has a launched a number of surveillance and research projects in the U.S. and with collaborators in other countries. On the domestic front, CDC has implemented the U.S. Zika Pregnancy Registry, an active surveillance network to prospectively enroll and follow pregnant women and their infants; a complementary registry has also been implemented in the Commonwealth of Puerto Rico. Both systems enroll pregnant women and infants with laboratory evidence of Zika virus infection, and prenatally and perinatally exposed infants. Individuals are enrolled into the registry by health departments or directly from health care providers. In Puerto Rico active surveillance is underway based on medical record abstraction. Data on the pregnant women will be collected at a number of points in pregnancy and on the infants at birth and at 2, 6, and 12 months of age, with follow-up to age 3 years in Puerto Rico. Clinical information will include attributes of the mother's health and Zika virus testing during pregnancy, results of fetal and newborn evaluations, and medical and developmental information on the infant. Emerging data from the registries will be used to inform clinical recommendations and public health actions. CDC is also enhancing its ongoing state-based birth defects surveillance programs to identify, in a real-time manner, any infant born with microcephaly or other Zika-related birth defects. In the international arena, CDC is engaged in several research and surveillance activities in Central and South America. Most notably, we are collaborating with the Secretary of Health of Paraiba state, Brazil and the Brazilian Ministry of Health on a case-control study of microcephaly to further characterize the association with Zika virus infection and the clinical phenotype of fetal Zika infection. We are also collaborating with the Instituto Nacional de Salud in Colombia to enhance their national surveillance of Zika virus disease among pregnant women and to implement in-depth monitoring of pregnant women in selected regions of Colombia with high reported incidence of Zika virus disease. A description of CDC activities and collaborations will be presented, including current status and anticipated outcomes.

Enhancing Zika virus Preparedness and Response in New Orleans, Louisiana, USA.

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~~Aedes aegypti and Aedes albopicus are found in New Orleans in abundance and present challenges for control due to the City's highly urbanized environment, abundant vegetation, high container indices (including tires), high number of blighted or substandard housing, and limited property accessibility. Despite the seasonality, abundant rain and warm temperatures provides a suitable habitat for these mosquitoes. New Orleans has robust tourism and port industries which brings travelers and cargo from all over the world, increasing the chances for Zika virus introduction. The City of New Orleans has created a framework of partner agencies (public and private) to enhance the ability to conduct mosquito control for a travel or mosquito-borne case of Zika virus. Sustainable Aedes spp. control is expensive and requires significant resources and a scientific approach to maximize efficacy and direct a targeted response. The City of New Orleans Mosquito and Termite Control Board (NOMTCB) utilizes an integrated pest management approach incorporating biological, chemical control as well as community involvement. An analysis of the mosquito control program, including equipment, staffing needs, and available supportive agencies and companies was conducted. Review of public health code was conducted to determine how to decreased the time to perform inspections, source reduction, and treatments. A written plan was created the to provide guidelines for the City of New Orleans describing an integrated approach to disease control, to incorporate operational research as a framework for evidence-based decision-making, and to provide health information to the public. Webinars, workshops, and seminars have been given to educate stakeholders prior to cases in order to enhance capacity and unify the messaging to the public. Prevention and mitigation of Zika virus in New Orleans will rely on collaborative efforts of many agencies and private business.

Surveillance and Container Assessments in Residential Environments for the Prevention of Mosquito-Borne Viruses in New Orleans, Louisiana

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The emergence of arboviral epidemics like Zika underscore the importance of public health practitioners to understand local transmission potential including practices of residents and abundance of competent vector populations. Large urban populations of *Aedes aegypti* and *Ae. albopictus*, vectors of Zika virus, are present in New Orleans, Louisiana and the potential for introduction by a viremic individual is of great concern. The New Orleans Mosquito Control Board utilizes an integrated pest management approach to mosquito control including door-to-door educational campaigns, source reduction and adulticide applications. This current study assessed the knowledge, attitudes and practices regarding mosquito-borne diseases among New Orleans residents and identified frequent mosquito breeding habitats in residential environments. Residents reported indicators of exposure including that mosquitoes were a problem in their yard (63.2%), spending time outside in evening daily (37.8%), and being bitten by mosquitoes frequently (45.9%). Precautions taken against mosquitoes was highly variable, including repellant use which ranged from frequently (25.8%) to never (28.2%). Property inspections in November and December 2015 yielded an average of 1.4 water-holding containers per residence and a House Index of 31.8. Of the 115 containers surveyed, 36.5% were positive for mosquito larvae and 13.9% for pupae. The most common mosquito species was *Aedes aegypti* (85.9%); far less common was *Culex quinquefasciatus* (11.3%) and *Ae. albopictus* (3.3%). Additional surveys are planned for April-May 2016 and adult mosquito surveillance will be conducted using BG-Sentinel traps. Collected mosquitoes will be tested for virus by RT-PCR. The intention of these surveys is to produce tailored educational outreach materials. It is essential to identify, educate, and eliminate residential mosquito breeding locations for *Ae. aegypti* on a community-wide level. The long-term control of arboviral diseases is only possible through an integrated public health approach, rapid case identification, and sustainable vector control and community involvement strategies.

Zika Virus Infection in Pregnant Women in Rio de Janeiro Preliminary Report

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~~Background Zika virus (ZIKV) has been linked to neonatal microcephaly. To characterize the spectrum of ZIKV disease in pregnancy, we followed patients in Rio de Janeiro to describe clinical manifestations in mothers and repercussions of acute ZIKV infection in fetuses. Methods We enrolled pregnant women in whom a rash had developed within the previous 5 days and tested blood and urine specimens for ZIKV by reverse-transcriptase-polymerase-chain-reaction assays. We followed the women prospectively and collected clinical and ultrasonographic data. Results A total of 88 women were enrolled from September 2015 through February 2016; of these 88 women, 72 (82%) tested positive for ZIKV in blood, urine, or both. The timing of acute ZIKV infection ranged from 5 to 38 weeks of gestation. Predominant clinical features included pruritic descending macular or maculopapular rash, arthralgias, conjunctival injection, and headache; 28% had fever (short-term and low-grade). Women who were positive for ZIKV were more likely than those who were negative for the virus to have maculopapular rash (44% vs. 12%, P=0.02), conjunctival involvement (58% vs. 13%, P=0.002), and lymphadenopathy (40% vs. 7%, P=0.02). Fetal ultrasonography was performed in 42 ZIKV-positive women (58%) and in all ZIKV-negative women. Fetal abnormalities were detected by Doppler ultrasonography in 12 of the 42 ZIKV-positive women (29%) and in none of the 16 ZIKV-negative women. Adverse findings included fetal deaths at 36 and 38 weeks of gestation (2 fetuses), in utero growth restriction with or without microcephaly (5 fetuses), ventricular calcifications or other central nervous system (CNS) lesions (7 fetuses), and abnormal amniotic fluid volume or cerebral or umbilical artery flow (7 fetuses). To date, 8 of the 42 women in whom fetal ultrasonography was performed have delivered their babies, and the ultrasonographic findings have been confirmed. Conclusions Despite mild clinical symptoms, ZIKV infection during pregnancy appears to be associated with grave outcomes, including fetal death, placental insufficiency, fetal growth restriction, and CNS injury. (NEJM March 4, 2016 DOI: 10.1056/NEJMoa1602412). From the analyzed period to April 2016 data will be updated.

The use of copepods as biological control for *Aedes aegypti*

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Cyclopoid copepods are tiny crustaceans which consume smaller aquatic animals including first instar *Aedes aegypti* and *Ae. albopictus* mosquito larvae. Copepod species vary widely in their capacity to consume mosquito larvae. One cyclopoid species, *Mesocyclops longisetus*, has been proven to be a voracious larval predator and survives well in extreme conditions including high temperatures. Mass production of these species is relatively inexpensive, colony maintenance requires minimal labor and copepods can be introduced easily into mosquito breeding sites with a conventional sprayer. Previous studies in New Orleans have demonstrated the capacity of *Macrocyclops albidus* to eliminate *Aedes* larvae in discarded waste tires. Programs in Vietnam and Honduras also demonstrated the successful elimination of *Ae. aegypti* in water storage containers including drums, vases and tanks and were also socially acceptable to the community. Once introduced, long-term populations can be maintained with only periodic monitoring and replacement if containers dry out or if water is removed. As part of NOMTCB's integrated pest management (IPM) approach to *Aedes* mosquito control copepods offer a biological and sustainable tool in historically difficult to manage containers.

Imported Zika Virus Outbreak in French Guiana: news insights on epidemiology and diagnosis

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Background:

On December 15th 2015, the first imported Zika virus (ZIKV) case was confirmed on Kourou in French Guiana, in a military community exposed to ZIKV in Surinam during two weeks. We conducted an investigation to i) take in charge all the patients, ii) realize a biological, clinical, serological and virological follow-up of each infected people, iii) describe epidemiological features of the outbreak and iv) evaluate the entomological situation and avoid any secondary case.

Methods:

On December 16th and 24th 2015, two rounds of active case detection were realized in the other 135 militaries by ZIKV RT-PCR on blood and urine samples, MAC and GAC ELISA for detection of IgM and IgG antibodies against ZIKV and Dengue virus (DENV). Titers of neutralizing antibody to ZIKV and DENV 1 to 4 were also determined. On January 14th 2016, everybody was submitted to a last round for serological analysis. Strict vector control measures, isolation of patients and individual protection against mosquito bites were ordered. In parallel, from December 16th to 21st 2015, entomological investigations were conducted inside the military camp to collect adult mosquitoes and test them by ZIKV RT-PCR.

Results:

A total of 12 ZIKV infections were confirmed (10 by both virological and serological tests, all positive serum samples for ZIKV IgM antibodies were positive for ZIKV neutralizing antibodies and negative for DENV 1 to 4 neutralizing antibodies). 58% were symptomatic and 42% asymptomatic. The overall attack rate for ZIKV infection was 9%. The global incidence rate of ZIKV infections was 6 per 1000 persons per day (CI95% [2.2-13.1]). The median duration of the maximum period of incubation for ZIKV disease was 10 days (range, 7 to 15). The median duration of ZIKV RNA detection in urine samples was of 14 days after onset (range, 9 to 19). ZIKV IgM antibodies seroconversion appeared at day 5 after onset. No mosquito was found infected by ZIKV.

Conclusions:

Our findings provide really new features in epidemiology and diagnosis of ZIKV infections. With a strict application of vector prevention and control measures, no secondary ZIKV case was reported.

Evaluating aerial ultra-low volume (ULV) adulticiding applications for *Aedes spp.* control

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Urban populations of *Aedes aegypti* and *Aedes albopictus*, potential vectors of both chikungunya and Zika viruses, are present in New Orleans, Louisiana in abundance. To control both mosquito species, the City of New Orleans Mosquito and Termite Control Board (NOMTCB) utilizes aerial adulticide missions as an important tool in their integrated pest management (IPM) program. During the summer of 2015, an ultra-low volume (ULV) aerial application of Dibrom® (naled; AMVAC Chemical Corporation, Newport Beach, CA) at 0.5 oz/acre was tested for efficacy against laboratory-reared, field-derived caged *Aedes* mosquitoes. Paired cages were placed in open and sequestered areas, including under raised homes and in dense vegetation, in two urban neighborhoods with high human density and abundant mosquito populations. Aerial applications against caged *Aedes aegypti* in open locations resulted in 90.1-90.7% average mortality and 81.6-97.9% in sequestered locations. *Aedes albopictus* mortality was 73.6%-99.6% in open locations and 66.3%-92.0% in sequestered locations. In addition, Tinopal® fluorescent dye (BASF Corporation, Florham Park, NJ) was mixed with Dibrom® and fluorescent droplets were captured utilizing rotating aerosol droplet samplers (John W. Hock, Gainesville, FL) and Teflon®-coated slides. Droplet analysis was conducted using Dropvision® (Leading Edge Associations, Inc., Fletcher, NC). Fluorescent droplets were present in the treatment areas only. Droplet collections were low, despite high mortality. This work demonstrates that aerial adulticide applications can rapidly reduce *Aedes* populations in outdoor open environments and cryptic sequestered resting sites. However, it is important that insecticide resistance studies are conducted routinely to determine the susceptibility of the mosquito population to the insecticide in use. The NOMTCB will continue to utilize aerial ULV adulticide applications against *Aedes* species in partnership with other intervention strategies including biological control and community involvement to mitigate abundant mosquito populations and reduce risk of vector-borne disease.

Descriptive and prospective study of Zika virus disease in Army forces in French Guiana (ZIFAG)

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A clinical research project on Zika virus (ZIKV) disease entitle "Descriptive and prospective study of Zika virus disease in Army forces in French Guiana (ZIFAG)" has just started and has been initiated by Military Health Service in collaboration with Pasteur Institute of French Guiana. This longitudinal cohort study aims to:

1) Describe clinical and biological characteristics of ZIKV disease in 100 patients during its acute phase and to evaluate its consequences on the quality of life during the first year after the onset of symptoms.

2) Determinate in all the patients the ZIKV serological diagnosis window by the kinetic study of ZIKV IgM and IgG antibodies responses in order to precise the calendar of pregnant women serological follow-up (because of asymptomatic infections).

3) To evaluate in 30 patients the contagiousness duration in capillary blood compartment (infectiousness for *Aedes aegypti* mosquitoes) in order to precise the duration of isolation of ZIKV patients under bednets.

4) To estimate in 20 patients the duration of infectiousness of semen in order to precise the period of condoms use to avoid sexual transmission.

Inclusion criteria comprised to be affiliated to a social security system, to be > 18 years old and to be voluntary to participate to the study and to sign an individual written consent. The non-inclusion criteria is pregnancy.

When a patient presents a cutaneous and two other symptoms (fever, asthenia, myalgia, arthralgia…), ZIKV RT-PCR is done on blood and urine samples, in case of positivity, he is included in the following protocol:

- Standardized physical examination at the first visit then D3 after onset, D5, D7, D15, D21, D28, M2, M3, M6, M9, M12 + validated standardized auto-questionnaires about quality of life since M1 until M12.
- Laboratory tests at the first visit until normalization if abnormalities.
- Venous blood sample at the first visit then D3 after onset, D5, D7, D15, D21, D28, M2, M3, M6, M9, M12.
- Capillary blood sample at the first visit then D3 after onset, D5, D7.
- Semen sample every 15 days until ZIKV RT-PCR negativity.

In-depth analysis of the human centrosome proteome reveals novel factors associated with innate immune signaling and development

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Several genes underlying primary microcephaly associate with mutation in centrosomal proteins making this group of proteins potential host factor candidates that might explain the fetal neurological complications associated with Zika virus infection. To facilitate the identification of a potential link between centrosomal proteins and viral diseases, we performed and in-depth analysis of the human centrosome proteome using quantitative proteomics. Using these methods, we have previously reported novel centrosomal proteins, including Cep135, Cep152 and Cep215 now associated with primary microcephaly (Nature, 2003). Motivated by the resulting mass spectrometry- and microscopy-based proteomics data, we speculate whether centrosomes function as solid-state signaling platforms for innate immune signaling. Centrosomes have an established role in immune responses by assembly of the immunological synapse in cytolytic cells for polarized delivery of lytic granules to eliminate infected cells. In contrast, an active role of centrosomes during innate immune responses has received comparatively little attention although centrosome integrity is often perturbed when pathogens intercept or subvert the microtubule and cell cycle regulatory system for trafficking and life cycle control. In support of this role, we identified novel pericentriolar-associated proteins, including adapter proteins, deubiquitylases, and members of the tripartite motif (TRIM) family of E3 ubiquitin-protein ligase subunits known to negatively regulate innate immune responses. We further identified the innate immune adapter protein flightless-1 (FLII) as a novel centriolar satellite protein. Upon intracellular pattern recognition receptor activation, FLII relocalized from centriolar satellites to small cytoplasmic structures, or independently of the centriolar satellite pool, assembled larger structures defined by the interaction partner LRRFIP2 and inflammasomes marker. We propose that these compartment-specific mechanisms help to control the production of interferons and proinflammatory cytokines under normal conditions and during innate immune responses.

Exploration of a study design to estimate male-to-female sexual transmission of Zika virus

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There are a number of cohort studies of pregnant women recruited when they have clinical signs suggestive of Zika virus infection. We propose a family-group extension to such studies in which clinical samples are collected as soon as possible after recruitment of the primary case (the pregnant woman with clinical signs) from household members and the primary male sexual partner of the primary case (we refer to both household members and the primary male sexual partner as being members of the "family group"). Indeed, there is no requirement for the primary case to be pregnant. The design works equally well for all women willing and able to identify a primary male sexual partner.

We propose a simple transmission model including background vector-borne transmission, within-household vector-borne transmission and male-to-female (m-to-f) sexual transmission from the primary male sexual partner to the primary case. Using data simulated using this transmission model, we demonstrate the statistical power to detect statistically significant m-to-f sexual transmission as a function of the number of family groups tested (sample size) and the sensitivity of the diagnostic test used for family group members. Allowance for incomplete diagnostic sensitivity is important because it may not be possible to recruit family group members if both blood and urine samples are required; thus, we consider fully accurate diagnosis of family group members and where diagnosis of family group members is based only on urine samples. We demonstrate that this study design is powerful to detect m-to-f sexual transmission both using the proposed transmission model used to simulate the data and using a simple sign test, although the latter does not provide an estimate of the m-to-f transmission probability.

The transmission model used in the first instance assumes that all family group members are subjected to the same level of background vector-borne transmission and that all household members (which may or may not include the primary male sexual partner) are subjected to the same level of within-household vector-borne transmission, regardless of gender, age, etc. These assumptions can be loosened, but at a cost of reduced statistical power for a given sample size.

Detection of Zika virus rapidly, economically, locally and reliably without the need for a laboratory using Isothermal amplification

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Zika virus (ZIKV) is an emerging arbovirus which can cause severe neurological disorders, spreading rapidly through the America, a public health emergency of international concern. Current diagnostic tests are inadequate, expensive and unavailable where they are needed most. We propose to develop, optimize and deploy an affordable, reliable, easy to use, rapid, and robust kit for rural and low-income use for the detection of arboviruses, both on human samples for diagnosis as well as on mosquitoes for surveillance purposes. The kit will utilize isothermal amplification techniques coupled to a smartphone app and sensor to collect and disseminate acquired data, providing new types of useful data for infectious disease monitoring, modeling, and prediction. Kit will be widely deployed, including to communities and schools, for vector surveillance, public engagement, and educational purposes.

Preparing Mexico for the Zika epidemic

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The first case of Zika virus infection was reported in Mexico in November 2015. However, the outbreak has been confined to a few cases (200 laboratory confirmed cases) since the rainy season has not begun. The rainy season will start in May and the peak of the epidemic is expected in August-September. The preparation of the country for this epidemic has included: vector control, health promotion to prevent mosquito reproduction and mosquito bites, epidemiologic and laboratory surveillance, and health care services preparation.

The Mexican Institute of Social Security (IMSS), the largest health care provider for the formal sector, will participate with the international community to understand this disease with the following activities: epidemiologic surveillance, clinical and epidemiologic research and estimation of the burden of this disease.

Epidemiologic surveillance will include 1500 IMSS clinics that take care of 40 million affiliates around the country. These clinics will registry all symptomatic cases that report to the clinic including Dengue, Chikungunya and Zika. All pregnant women confirmed with Zika infection will be followed up to delivery.

Clinical and epidemiologic research will include: a cohort of pregnant women, a case control study of Guillain-Barré syndrome and a case control study of microcephaly in the most affected states.

The estimation of the disease burden will include our system of total temporary disability related to economic subsidies to formal workers in addition to the fever syndrome epidemiologic surveillance. A previous estimation of Chikungunya infection based on the algorithm for laboratory confirmation of symptomatic cases and the mix with dengue in clinical practice, has allowed us to estimate that each confirmed case of Chikungunya represents 100 symptomatic cases.

Although the magnitude of the epidemic in Mexico is uncertain, if the outbreak resembles the attack rates of other countries, IMSS will take care of more than 12,000 symptomatic infected pregnant women and more than 700 cases of Guillain-Barré syndrome.

Neural stem cell infection by Zika virus in the mouse developing neocortex

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Neurotropic flaviviruses are human pathogens responsible for numerous viral encephalitic diseases. West Nile and Japanese encephalitis flaviviruses infect the adult brain and are also responsible for congenital infections that impact fetal brain development. Zika virus is also a neurotropic flavivirus but seems rarely responsible for neurological diseases in adults. However, Zika virus infection was recently linked to microcephaly, a disease causing devastating congenital disorders and characterized by a reduced size of the cerebral cortex which is due to reduced neurogenesis or increased cell death. It is currently unclear which specific cell types may be infected in the developing neocortex by Zika virus and what the consequences of infection may be on its development.

In order to address these questions, we have developed an assay to infect mouse E15 embryonic brain slices, and performed infections with Zika, West Nile and dengue (serotype 4) viruses. We show that mouse neocortex is able to support viral replication of Zika and West Nile viruses, but not of the non-neurotropic dengue 4 virus, after 12, 24, 36 and 48h post-infection, as demonstrated by viral titration. Using immunostaining of flavivirus E glycoprotein and cell fate markers, we asked which cell types were preferentially infected by Zika and West Nile viruses in the developing neocortex. While West Nile virus shows a preferential tropism of infection for neurons, Zika virus infection was strongly biased towards the neural stem cells.

We are currently examining the effect of Zika virus infection on neural stem cell proliferation and induction of apoptosis. Altogether, our work supports a model whereby preferential infection of the neural stem cells by Zika virus in the developing neocortex may lead to reduced neuronal production and microcephaly. It further establishes the mouse developing neocortex as a powerful model system to investigate the link between Zika virus and microcephaly.

The highly structured flaviviral 3'UTR determines transmission of flaviviruses by mosquitoes

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Flaviviruses are of high importance for human health and are collectively responsible for millions of reported cases of infection annually. Most of the clinically relevant flaviviruses are mosquito-borne, such as the highly pathogenic dengue virus, the emerging Zika virus and West Nile virus. The flavivirus genome is flanked by 5' and 3' untranslated regions (UTRs) that form complex secondary and tertiary RNA structures. Although much is known about the role of the 3' UTR in flavivirus pathogenesis in mammalian hosts, its function in the mosquito host is largely unknown. Recent research showed that a specific RNA structural element (called stem loop II or SL-II) within the variable domain of the 3'UTR is highly preserved during mosquito infection while other secondary RNA structures accumulate mutations, suggesting an important role of SL-II in flavivirus transmission. We investigated the function of SL-II during West Nile virus infection of *Culex pipiens* mosquitoes and evaluated its role in vector competence. A West Nile virus mutant with mutations in SL-II was generated that displayed significantly decreased infection and transmission rates in mosquitoes when administrated via the blood meal. However, the SL-II mutation did not affect the transmission or infection rates after intrathoracic mosquito injection, thereby identifying SL-II as key driver for flaviviruses to overcome the mosquito midgut infection barrier. This is the first report to describe an essential biological function of the flaviviral SL-II RNA structure within the 3'UTR in the context of transmission by the mosquito vector, which provides an explanation for its strict conservation in all flavivirus genomes.

Infection of *Aedes albopictus* and *Aedes aegypti* with Zika virus: perspectives for an emergence in Europe

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Diseases caused by arbovirus are a major public health challenge in many parts of the world. Despite intense research, vaccination or specific treatments are still needed for most of them. In order to control those diseases, a better understanding of host-vector-virus interactions is required. In particular, vector-virus interaction remains poorly understood although it could provide key information about the virus epidemiology, physiopathology or virulence. Zika virus (ZIKV) is an emerging arbovirus of the *Flaviviridae* family. ZIKV was first discovered in Uganda in 1947. Different species of mosquito from the *Aedes* genus are the vectors responsible for ZIKV infection in humans. Until recently, ZIKV outbreaks were irregular and self-limiting. The first large epidemic was reported from Yap Island in 2007 followed by an outbreak in French Polynesia in 2013. Brazil is the epicenter of the current ZIKV epidemic which is quickly disseminating across the Americas. *Aedes albopictus* is present in most European countries and may be the key to ZIKV emergence in Europe.

Our study focused on the interaction between *Aedes mosquitoes* and ZIKV (pf13 strain). For this purpose, we performed experimental infections of adults *Aedes albopictus (Nice, France)* and *Aedes aegypti (PAEA strain)* by ZIKV in our laboratory. Then, salivary glands and midguts were removed; the viral loads in those organs were analyzed by RT-qPCR and plaque assay. Thus, we were allowed to determine the dissemination of the arbovirus in the vector's organism during the first two weeks of the infection. We found that high viral replication was observed at day 5 post-infection (DPI) in midguts and at 10 DPI for salivary glands for both vectors. Although most salivary glands were found infected at day 10, only 10 to 30% of saliva collected at D14 were found infected. These results may indicate either that the extrinsic incubation period is longer for these mosquitoes or that ZIKV may have difficulties in crossing the transmission barrier emphasizing on the need of comparative proteomic approaches in order to better understand vector/virus interactions. The next step will then be to perform a *label-free* LC-MS proteomic analysis of midguts and salivary glands of both *Aedes* mosquitoes in the presence and absence of ZIKV.

Emerging mosquito-borne viruses threats to Europe: Entomological preparedness and risk assessment for emerging arboviral diseases in Greece including Zika, dengue and chikungunya

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The perception that the temperate climate of Europe provided a risk-free environment for mosquito-borne tropical diseases has ended. Autochthonous cases have been recorded of dengue in 2010 for France and Croatia, of chikungunya in 2007 for Italy and in 2010 for France, and of West Nile virus in 2010 and 2011 from the Southern Mediterranean basin to Austria, Hungary and Southern Russia. More recently, the explosive Zika virus epidemics in South/Central Americas and the Caribbean have led to many imported cases reported in several European countries. It has now become clear that Europe is at risk for epidemics of emerging mosquito-borne diseases. Europe, particularly Greece and other territories of the Mediterranean region, is vulnerable and receptive for the emergence of mosquito-borne diseases. In Greece, this vulnerability and receptivity are increased by the massive flows of tourists and refugees by sea and land, the presence of the mosquito vector *Aedes albopictus*, the susceptible human populations and the higher year-round temperature and humidity that facilitates both vector abundance, vector-host contact and pathogen transmission. The occurrence of autochthonous cases of dengue and chikungunya in France, Italy and Croatia in areas where the only known vector mosquito species is *Aedes (Stegomyia) albopictus* Skuse (1894) reinforces the importance of this vector in the transmission of arboviruses in Europe. So far, *Ae albopictus* has not been found naturally infected with either dengue or chikungunya in Europe. Nonetheless, Mediterranean *Ae albopictus* mosquito populations from France and Italy were high susceptible to chikungunya virus under laboratory-controlled conditions. In Greece, little is known about the populations of *Aedes albopictus*, apart from fortuitous sites of occurrence and a local longitudinal study in the capital Athens. Due to the geopolitical importance of Greece as a port of entry for emerging arboviruses, we are proposing to investigate representative Greek populations of *Ae albopictus* for entomological parameters such as: estimation of population size; seasonal variation; intraspecific genomic variation among populations; distribution prediction; natural infection by flaviviruses; insecticide resistance and vector competence for arbovirus strains. Basic entomological studies on Greek populations of *Ae albopictus,* along with *Aedes albopictus* vector competence, are needed in order to fully assess the risk of emerging arboviruses and to effectively contribute to integrated vector control strategies to prevent the emergence of arbovirus infections in the Mediterranean and other receptive areas of Europe.

The role of telemedicine in response to the Zika virus outbreak in Brazil

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Objective: Telemedicine delivers healthcare from a distance and is well implemented in Brazil. The outbreak of Zika virus (ZIKV) in Brazil was declared a public health emergency of international concern (PHEIC) by the World Health Organization (WHO) in February 2016. None of the PHEIC response documents included telemedicine. The objective of this review is to assess the potential role of telemedicine during the ZIKV outbreak occurring in Brazil.

Method: The WHO strategic response framework, the joint operational plan, and all interim guidance related to ZIKV outbreak PHEIC were compared with evidence from a literature review regarding telemedicine.

Findings: Evidence was available on telemedicine in relation to surveillance, care and research topics. Concerning surveillance, the results reported only on telemedicine providing support of laboratory activities. For the care of those affected, no results were available on telemedicine in the diagnosis and management of microcephaly and Guillain-Barré syndrome specifically. Telemedicine in gynaecology and obstetrics was reported with cardiotocograph, ultrasound, abortion, and breastfeeding. Telemental health was also reported as effective. One study used the database of a telemedicine network in Brazil for an epidemiological research purpose.

Conclusion: To our knowledge, this review was the first to assess available evidence on telemedicine in the context of PHEIC relating to the ZIKV outbreak. Telemedicine is reported in the literature to be effective in clinical situations that have to be faced during the current ZIKV outbreak and might be an interesting tool to support its control. More research on the topic appears to be necessary.

ZIKA virus replication in human placenta

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Zika virus (ZIKV) is an emerging arbovirus of the *Flaviviridae* family transmitted to humans by mosquito bites and sexual route. ZIKV caused a major outbreak in French Polynesia in 2013/2014 and has since spread in 2015 to the Americas, notably in Brazil. Most cases of zika infection in otherwise healthy adults are asymptomatic or pauci-symptomatic, with fever, rash, arthralgia, and conjunctivitis. ZIKV infection has recently been associated with severe fetopathy, including microcephaly (Brasil et al., N Engl J Med. 2016, in press). Placental calcifications are observed in ZIKV-infected pregnant women and ZIKV is also present in the amniotic fluid and brain of fetuses with microcephaly (Calvet et al., Lancet Infect. Dis., 2016, in press; Mlakar et al., N Engl J Med. 2016, in press; Sarno et al., PLOS Negl Trop Dis, 2016). Together, these data strongly suggest that ZIKV has ability of ZIKV to cross the placental barrier and induce fetopathy.

Here, we have assessed the ability of ZIKV to infect human placental explants and human trophoblast cell lines, as compared to Chikungunya virus (CHIKV), another emerging arbovirus which is not able to infect the placenta and is exclusively transmitted vertically from viremic mother to their baby during parturition via placental breaches. We infected third trimester human placental explants with either ZIKV or CHIKV, and viral load in placental tissues and supernatants were determined daily from day 1 to day 4 post-infection. We found that ZIKV replicates in human placental explants, as shown by viral titers in placental tissues and supernatants, while, as expected, CHIKV does not. The identification of the cells targeted by ZIKV in human placental explants is currently investigated. In addition, a human trophoblast cell line was shown to be susceptible to ZIKV infection, but resistant to CHIKV infection. These preliminary data suggest a direct ZIKV tropism for human placenta. The relevance of these results will be assessed by detection of ZIKV in human placental biopsies of infected pregnant women. Studies in animal models susceptible to ZIKV will help decipher how and when this virus crosses the placental barrier.

Unbderstading the placetnal phase of ZIKV infection is required to understand its vertical transmission and develop potential preventive and therapeutic strategies against this emerging teratogenic arbovirus.

The International Severe Acute Respiratory and emerging Infection Consortium (ISARIC) has been working with partners in support of the research response to the ZIKV outbreak

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In early December 2015, ISARIC's Fiocruz-based regional focal point in the Americas informed us of the rise in cases of microcephaly, which might be associated with the Zika virus outbreak in Brazil. In collaboration with the Global Health Network we set up a website www.zikainfection.org to help ensure dissemination of up to date information and for a place where scientists could share tools and protocols – identified as an urgent need by our focal point.

Addressing another urgent need, ISARIC, working in partnership with PREPARE Europe, sought to put together research tools that aimed to capture a minimal data set regarding the natural history of the disease. This resulted in a series of Case Report Forms for microcephalic new-borns and pregnant women. Currently, we are aligning the CRFs with those used by a sister network: REACTing. This is an example of the move towards harmonisation of tools seen in this Zika outbreak response. ISARIC and PREPARE's CRFs are being used in a number of countries in the affected region and we will send a Master's student to Brazil to carry out an end-user assessment to further validate and improve them and the tool-development process in general.

Through our regular teleconference calls with key stakeholders to the outbreak e.g. Institut Pasteur we have formed small project groups that focus on outcomes, data sharing and laboratory algorithms for researchers.

To further Zika collaboration and cooperation with public health bodies, we are exploring potential areas of collaboration with the International Association of National Public Health Institutes (IANPHI), and other key stakeholders.

We see these initiatives as activities which can be built upon for responses to other disease outbreaks. The spirit of global collaboration is so encouraging and should continue to lead to the harmonisation of tools and protocols to allow for data sharing in the near future.

Miniaturized peptide based differential serology

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Viruses like *Variola major* and *Ebola* are extremely hazardous to mankind, although recombinant proteins or synthetic peptides of their sequences are safe to produce. A few virus genera, like Flaviviruses, only differ in a few amino acids of their envelope proteins. Their serological differentiation using whole virus lysate or single recombinant antigens is a challenging task due to potential cross-reactivity. Therefore, a peptide-based serology with the ability to distinguish between those single amino acid changes is clearly required. Modern peptide microarrays represent tools for fast screening and differential serology. We have manufactured tiling arrays with minimal offsets (1-3 amino acids) with up to 10.000 peptides per slide that address different proteins or whole polypeptide chains of viruses (like *Zika virus*). Screening of antibody targets, including cross reactivity testing of closely related family members (e.g. *Herpes simplex viruses* (HSV-1+2) vs. *Herpes simian B virus* (BV)), can be achieved with a minimum of sample amount (~1µl) in a single test run.

We have demonstrated that the resulting epitope-mapping of BV infection in macaques showed a diverse pattern to known epitopes of HSV infections in humans as well as to known monoclonal antibody targets on the two glycoproteins B and D. Within this single study we were able to detected overall 18 epitope regions, 17 of which had not been described earlier. Ongoing studies in our lab comprising e.g. *Hepatitis C virus* (another member of *Flaviviridae*) or mapping of monoclonal antibodies against different Orthopoxviruses (unpublished data).

Such serological fingerprints will provide possible markers for differentiation, which afterwards can be translated to standardized tests (e.g. Luminex) and/or new vaccine strategies. We would like to apply our technical knowledge in the field of peptide-based diagnostics to distinguish *Zika virus* infections from other closely related Flavivirus genus members like *Dengue* (1-4), *West Nile* and *Yellow fever viruses*.

Isolation of infective Zika virus from urine and saliva of patients in Brazil

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Since May 2015 a very intense widespread outbreak of Zika virus (ZIKV) has been reported in Brazil with an increase in Guillain-Barré syndrome and microcephaly cases. As an emergent disease, several aspects of the infection still have to be revealed. Since the French Polynesia outbreak, urine and saliva have been established as useful tools in ZIKV diagnosis of patients in acute phase. However, no evidence regarding the infectivity of ZIKV particles present in saliva and urine has been obtained at that time. For this reason, we aimed to investigate the presence of ZIKV infective particles in these body fluids. In this study, urine and saliva samples from patients from Rio de Janeiro presenting rash and other typical Zika acute phase symptoms were inoculated in Vero cell culture and submitted to specific ZIKV RNA detection. Five ZIKV isolates were accomplished, three from urine and two from saliva specimens. The urine viral loads were higher when compared to the saliva ZIKV, supporting the concept that urine is a better source for ZIKV molecular diagnostic. Two of isolated strains, from different patients, one derived from urine and the other from saliva had their complete genome of both ZIKV isolates elucidated. Phylogenetic analysis revealed similarity with strains previously isolated during the South America outbreak. The detection of infectious ZIKV particles in urine and saliva of patients during the acute phase may represent a critical factor in the spread of virus. However, this epidemiological relevance of alternative non vector ZIKV transmission routes needs further investigation.

Full-length genome sequencing and analysis of 3 ZIKV strains on an Ion Torrent PGM Sequencer

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Background: Zika virus (ZIKV), an emerging arbovirus, was first isolated in 1947. In October 2013, French Polynesia (FP), South Pacific, suffered the largest ZIKV outbreak ever reported, with subsequent ZIKV outbreaks in 2014 in the Pacific region. No severe diseases resulting from ZIKV infection had previously been reported but, during the FP outbreak, severe neurological complications have been described. There is limited information about the genotype circulating in the country causing such conditions, and its potential to acquire varying competence to host-adaptation. Therefore, establishment of alert systems and promotion of awareness in the case of ZIKV epidemics is necessary to anticipate the emergence of new or more virulent viral strains.

Objectives: Both the lack of data on this pathogen and its rapid emergence stress the necessity for more comprehensive genetics-driven studies in order to measure the ability and potential to host-adaptation of the virus, and to elaborate whether severe neurological manifestations may be associated to recent infection by ZIKV.

Methods and Materials: Viral RNA was extracted from infected cell culture supernatants following nonspecific amplification (Whole Transcription Amplification). The full-length genome of three ZIKV strains was obtained by High Throughput Sequencing (HTS), using an Ion Torrent PGM.

Results: We successfully sequenced the full genome of three ZIKV virus strains, one from the FP outbreak, and the 2 others from former African isolates (1980 and 1991). Data analysis for the FP strain revealed low variability compared to a genome recently added to public databases, corresponding to a French imported case from FP, with the exception of a number of variable sites, evidencing genomic micro-evolution during outbreaks. Phylogenetic reconstruction confirms the samples clustered with respectively Asian and African genotypes.

Conclusion: We obtained genomic sequences for the newer Asia genotype FP ZIKV isolate, in addition to the 2 former Africa-genotype isolates, which will be committed to the scientific community, the analysis of which underlined the importance of genetic surveillance to emerging epidemic viral strains.

ZIKA VIRUS: The onset in Brazil

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A new challenge has arisen in Brazil with the emergence of ZIKA virus (ZIKV), another arbovirus in circulation. On March 26, 2015, patients at Santa Helena Hospital in Camaçari, a city distant 50 km from Salvador, capital of Bahia, Brazil were given a presumptive diagnosis of an acute viral illness by emergency department physicians. The illness characterized by maculopapular rash, fever, myalgias/arthralgia, conjunctivitis and with many patients that complain about an intensive rash in arms and legs and low fever (38,5 C). Dr Bandeira, a physician who was working in Santa Helena Hospital, consulted us if we can investigate this atypic non-dengue like illness. We accepted it and asked him to collect blood samples from patients with acute symptoms, no more than two or five days of illness. From about March 20 we started to search in serum the presence of other arbovirus: West Nile, Mayaro virus, Sant Louis virus including Dengue virus by conventional RT-PCR. However all were negative, there was only one more possibility to investigate: ZIKV. On March 26 2015, in Bahia we identified an outbreak to ZIKV for the first time in Brazil and Latin America from sera of patients with an illness characterized by maculopapular rash, fever, myalgias/arthralgia, and conjunctivitis. This result was immediately informed to the national health authorities (Ministry of Health, Brazil) and the presence of ZIKV (autoctones cases) in Bahia was confirmed by National reference centers. Nowadays, autoctones cases ZIKV are disseminated in all of our country.A new challenge has arisen in Brazil with the emergence of ZIKA virus (ZIKV), another arbovirus in circulation. On March 26, 2015, patients at Santa Helena Hospital in Camaçari, a city distant 50 km from Salvador,

Ethico-legal and societal issues and implications of emerging Zika virus epidemics complications and measures

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Background: Much of the fear and uncertainty around Zika virus (ZIKV) epidemics stems from potential association between ZIKV infected pregnant women and risk of their babies being born with microcephaly and other neurological pathophysiological on the embryo and fetus. But much remains unknown about transmission, diagnosis and long-term congenital pathogenesis and constellation of abnormalities including loss of fetus (miscarriage or prematurity, stillbirths) and/or severe birth defects. **Methods:** This explorative research survey aims at assessing the short and long term uncertainties of ZIKV epidemics effects and dichotomy between medical, ethical, legal, religious and families and proxy or informed consent in interventions explain the urgent needs of effective and efficient medical-legal programs and strategies such as rubella virus impact. **Results:** We documented challenges and issues including anticipating preventive and management care delivery to pregnant women and/or fetus, complications due to societal ethico-medical tenets, perceptions and practices. Potential unknown implications of Zika-related pathophysiological, emotional and societal consequences such as trauma, stress, depression, stigmatization and social withdrawal impacts on the affected populations and nationwide socio-demographic and economic outcomes. The local and global community has the responsibility to protect and promote people health, in maintaining the fundamental health principles in moral, medical and legal decision-making policies and interventions. Fostering health professionals' ethics and deontology within complex nature of decision making, individual and collective autonomy, fairness, protection of most vulnerable, proportionality, morality and dignity, integrity and beneficence that should not be confused and relegated by compassionate humanitarian assistance and support. **Conclusion:** The paper explores the potential medical and ethical-legal implications of ZIKV epidemics and interventions strategies on reproductive and mental health policies and measures. As well further research on Zika-related population-based ethico-medical and societal issues implications, especially maternal-child health require attention in rolling out future Zika vaccination programs in most affected countries and worldwide.

A modelling analysis of Zika transmission dynamics and associated microcephaly risk in Brazil

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In 2015, a significant increase in the incidence of microcephaly in newborns was reported in Northeast Brazil. An association between Zika infection and development of microcephaly during the first trimester of pregnancy has been suggested. However, the risk of microcephaly from infection is unclear. By fitting a transmission model to publicly available microcephaly incidence data, we assessed whether or not there was heterogeneity in Zika and microcephaly attack rates between Brazilian states.

We developed a deterministic compartmental model to account for Zika spread, including children, non-pregnant adults and adults in the first trimester of pregnancy. We assumed that the number of newborns with microcephaly was proportional to the number of infected first-trimester adults 6 months previously. We fitted the model generated incidence curves to microcephaly incidence data for each Brazilian state. We estimated values for the basic reproductive number (*R0*), the epidemic start time, probability of microcephaly given infection (*pm*) and baseline probability of microcephaly.

For each state that displayed epidemic-like dynamics, we were able to produce well constrained model fits. Our estimates for *R0* and *pm* varied between states. Mean and 95% CI estimates for *R0* were 3.23 (2.96-3.53), 2.97 (2.67-3.30) and 2.51 (2.14-2.89), and estimates for *pm* were 0.275 (0.250-0.303), 0.203 (0.180-0.227) and 0.0370 (0.0306-0.0454) in Pernambuco, Bahia and Sao Paulo respectively. Mean estimates for *R0* and *pm* across all states ranged from 1.64-4.06 and 0.0171-0.413 respectively. Our results suggest that there may have been substantial differences in attack rates between states. Estimated differences in *pm* may indicate either genuine differences in susceptibility and transmission, or systematic differences in reporting rates.

Our results highlight the importance of fully understanding the differences in risk of microcephaly between Brazilian states. A retrospective cohort of all newborn head circumference observations could be fit to the same model and may be able to distinguish between reporting bias and underlying biological heterogeneity.

Epitope analysis of Zika virus

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We have applied computational immunology approaches to analyze the predicted host- virus interface of Zika virus to determine predicted epitopes, changes in epitopes between African and Asian-American isolates of Zika, and the interface between predicted immune responses to Zika virus and to other co-endemic flaviviruses. We identify potential antibody mimic epitopes, indicative of anti-Zika antibodies which react with human proteins with neurologic functions. This apparent mimicry is consistent with the clinical signs of autoimmunity observed (Guillain Barré syndrome), as well as with the microcephalic and ocular lesions associated with Zika infection during pregnancy. A definitive link between Zika infection and GBS and microcephaly is still pending experimental and epidemiologic confirmation, however our analysis suggests that an antibody-mediated immunopathogenic pathway may compound the effects of viral replication. We will discuss the implications for vaccine and immunotherapeutic development.

ZikaRun: an integrative mother-infant inception cohort study to anticipate the introduction of Zika virus in the at-risk La Réunion island, Indian Ocean.

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Background. Zika virus (ZIKV), an arthropod-borne virus in the genus Flavivirus, spread by *Aedes* mosquito vectors, has become a serious threat for human populations living in epidemic settings and for international travelers, due to its highly probable ability to cause microcephaly and neurologic disorders, an epidemiologic situation which has conducted the World Health Organization to declare a public health emergency of international concern on February 1, 2016. Importantly, the presentation of the infection as a predominantly asymptomatic disease makes ZIKV difficult to control. In La Réunion island, where *Aedes albopictus* has gained superior competitive ability relative to *Aedes aegypti*, the interests of the scientific community and public health stakeholders have converged for preparing an integrative response to the possible emergence of the ZIKV. Perinatal transmission of ZIKV has been deemed the topic of interest and the cohort study the design of choice, owing to the need for quality data, internationally recognized experience on maternal-fetal transmission of Chikungunya virus, a decade for refining the surveillance system, and recent capacity building in clinical and translational research, enabling the possibility of new observations in this competitive area.

Primary objective. To assess the neurodevelopmental burden of congenital ZIKV infection.

Study design. (1) Mothers: non-randomized inception cohort with consecutive enrolments over a 4-month period; (2) Infants: randomized 2-year longitudinal follow-up over a 33 month-period (9+24).

Main outcome measure. Blind evaluation of Brunet-Lézine developmental quotient around two years of age.

Components. Task 1: Obstetrical risk. Task 2: Fetal risk. Task 3: Genetics. Task 4: Perinatal risk. Task 5: Pediatric risk. Task 6: Host-pathogen interactions. Task 7: socio-environmental risk, surveillance and prevention. Task 8: causality of risks.

Study size. (1) 3,600 mother-infant pairs. (2) 160 infants (120 infected, 40 at each trimester).

Conclusions. According to different scenarios, the expected number of new cases of primary microcephaly should be comprised between 12 and 32. The risk for small-for-gestational age infants should be multiplied by 2 and that of poor global neurodevelopmental performance by 3 in exposed children. The strength of this project will rely on its integrative approach including not only research but also surveillance and prevention tasks.