International Symposium on Zika Virus Research
Marseille - France, 4-6th June 2018

Auditorium Toga
Faculty of Medicine - La Timone

27, Boulevard Jean Moulin
13005 Marseille

Event organised by the EU funded Zika project

In collaboration with the three EU funded Zika projects

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The scientific programme

Monday June 4th, 2018

08.15 – 09.00 **Registration**

09.00 – 09.15 **Welcome note**
Xavier de Lamballerie, Inserm (ZIKAlliance coordinator)
Marion Koopmans, European Society for Virology - Erasmus Medical Centre - ZIKAlliance
Annelies Wilder-Smith, Umeå University (ZikaPLAN coordinator)
Carlo Giaquinto, PENTA Foundation (ZIKAction coordinator)
Frédéric Tangy, Pasteur Institute (ZIKAVAX member)

09.15 – 11.00 **Session 1: Surveillance, burden estimates, modelling**
Chair: Simon Cauchemez, Pasteur Institute - ZIKAlliance
Co-chair: Oliver Brady, London School of Hygiene & Tropical Medicine - ZIKAlliance

1. Zika in the Americas

Seroprevalence studies in Martinique/Guadeloupe (5')
Speaker: Pierre Gallian, Inserm - ZIKAlliance

Seroprevalence studies in Bolivia (5')
Speaker: Mariela Saba Villaroel, Inserm – ZIKAlliance*

High seroprevalence against Zika virus in Salvador, north-eastern Brazil limits the potential for further outbreaks (5')
Speaker: Andres Moreira-Soto, Charité University Berlin - ZIKAlliance*

Seroprevalence studies in Surinam (5')
Speaker: Thomas Langerak, Erasmus Medical Centre – ZIKAlliance*

Endemicity and emergence of arboviruses in Colombia: Insight from population based serological studies (5')
Speaker: Isabel Rodriguez-Barraquer, University of California/ Industrial University of Santander – ZIKAlliance*

Q & A (5')

2. Zika in Africa & Asia

Zika in Mali (5')
Speaker: Issa Diarra, Unité des Virus Emergents – ZIKAlliance*

Zika in Cameroon/Congo (5')
Speaker: Elif Nurtop, Unité des Virus Emergents - ZIKAlliance*

Zika in Burkina Faso (5')
Speaker: Nico Fischer, University of Heidelberg – ZIKAlliance*

Low Zika virus seroprevalence in Vientiane, Laos, 2003-2015 (5')
Speaker: Audrey Dubot-Pérès, Unité des Virus Emergents- ZIKAlliance

High baseline prevalence of microcephaly in Zika-epidemic and non-epidemic regions: data from sub-Saharan Africa, Asia, and the Antilles (10')
Speaker: Anna Louise Funk, Pasteur Institute – ZIKAlliance*

Q & A (5')

** Young researchers*

3. Modelling approaches to predict Zika epidemics at a local & continental scale

***Real-time assessment of healthcare requirements during the Zika virus epidemic in Martinique* (10')**

Speaker: Alessio Andronico, Pasteur Institute - ZIKAlliance

Modelling & predicting Zika circulation in Latin America (10')

Speaker: Oliver Brady, London School of Hygiene & Tropical Medicine – ZIKAlliance

Q & A (5')

4. Genetic & epidemiological analysis of Yellow Fever in Latin America to better understand the transmission cycle (10')

Speaker: Nuno Faria, University of Oxford – ZIKAlliance

Q & A (5')

Total: 105' (1h45')

Coffee Break

11.00 – 11.30 **Session 2: ZIKA: Clinical biology (part 1)**

Chair: Marion Koopmans, Erasmus Medical Centre - ZIKAlliance

Co-chair: Rosanna Peeling, *London School of Hygiene & Tropical Medicine* - ZikaPLAN

1. <u>Key-note lecture:</u> Zika virus diagnostics, the challenge! (15')

Speaker: Marion Koopmans, Erasmus Medical Centre – ZIKAlliance

2. The grim reality from a clinical researcher perspective (10')

Speaker: Vivian Avelino-Silva, University of Sao Paolo - ZIKAlliance

3. The diagnostic challenge of congenital Zika virus infection: lessons from TORCH pathogens (10')

Speaker: Bettie Voordouw, Dutch National Institute for Public Health & the Environment - ZIKAlliance

4. Glimpses of hope: towards more specific serology (10')

Speaker: Ernesto Marques, Oswaldo Cruz Foundation - ZIKAlliance

11.30 –12.25 ***Q & A (10')***

Total: 55'

Lunch break

12.25 – 14.00 **Session 2: ZIKA: Clinical biology (part 2)**

Chair: Marion Koopmans, Erasmus Medical Centre - ZIKAlliance

Co-chair: Rosanna Peeling, *London School of Hygiene & Tropical Medicine* -ZikaPLAN

5. Multiplex serology: new tools for complex problems (10')

Speaker: Jessica van Homwegen, Pasteur Institute – ZIKAlliance

6. Models for biobanking for rapid assessment of diagnostics in emerging disease outbreaks (10')

Speaker: Rosanna Peeling, London School of Hygiene & Tropical Medicine – ZikaPLAN

7. Identification of a putative unique immunogenic Zika NS2b epitode for differential diagnosis and surveillance (10')

Speaker: Isabelle Viana, Oswaldo Cruz Foundation – ZIKAlliance*

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14.00 – 15.10	***Q & A (5')***
	8. Studying hypotheses for antibody enhancement in a cohort context (10') *Speaker: Barry Rockx, Erasmus Medical Centre – ZIKAlliance*
	9. ZIKV cellular immune responses (10') *Speaker: Vincent Vieillard, INSERM – ZIKAlliance*
	10. CD8 T cell immunity to Zika virus in humans is shaped by prior DENV exposure and associated with an IFN gamma/cytotoxic signature (10') *Speaker: Daniela Weiskopf, La Jolla Institute- ZikaPLAN*
	Q & A (5')
	Total: 70' (1h10')
	Coffee Break
15.10 – 15.40	**Session 3: Vectors & vector control** *Chair: Anna-Bella Failloux, Pasteur Institute - ZIKAlliance* *Co-chair: Louis Lambrechts, Pasteur Institute - ZikaPLAN*
	1. Domestic *Culex* species & Zika vector transmission (15') *Speaker: Ricardo Lourenço-de-Oliveira, Oswaldo Cruz Foundation – ZIKAlliance*
15.40 – 17.40	***Q & A (5')***
	2. A new high-throughput tool to screen mosquito-borne viruses in Zika virus endemic/epidemic areas (10') *Speaker: Sara Moutailler, Anses – ZIKAlliance*
	Q & A (5')
	3. Global analysis of the virome in *Aedes aegypti* mosquitoes: arthropod borne-viruses & beyond (10') *Speaker: Roenick Olmo, Federal University of Minas Gerais – ZIKAlliance*
	Q & A (5')
	4. Has Zika burnt itself out of Brazil? Preparedness tools for the next outbreak (10') *Speaker: Eduardo Massad, University of Sao Paolo – ZikaPlan*
	Q & A (5')
	5. Potential of Aedes albopictus as a bridge vector at urban-forest interface in Brazil (7') *Speaker: Taissa Santos*, IRD – ZIKAlliance*
	6. Worldwide survey of *Aedes aegypti* susceptibility to multiple strains of Zika virus (7') *Speaker: Fabien Aubry*, Pasteur Institute – ZikaPLAN*
	7. Vector competence to Zika virus of mosquitoes from the Pacific region (7') *Speaker: Elodie Calvez*, Pasteur Institute New Caledonia – ZIKAlliance*
	8. Optimization of odour-baited trapping systems for the surveillance & control of *Aedes aegypti* in Paramaribo, Surinam (7') *Speaker: Tessa Visser*, Wageningen University – ZIKAlliance*

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	9. Identifying genomic changes associated with insecticide resistance in the dengue mosquito *Aedes aegypti* by next-generation sequencing (7') *Speaker: Julien Cattel*, CNRS – ZIKAlliance*
	Q & A (15')
	10. The Infravec2 Infrastructure Project: Providing Vector Researchers with No-cost Resources, Services and Facility Access (5') *Speaker: Eva Veronesi, University of Zurich – ZIKAlliance*
	Total: 120' (2h')
	Dinner at Fort Ganteaume (upon registration)
20.30	

** Young researchers*

Tuesday June 5th, 2018

08.35 – 10.30 **Session 4: Cohort Studies**

Chair: Thomas Jaenisch, University of Heidelberg - ZIKAlliance

Co-chair: Patricia Brasil, Oswaldo Cruz Foundation – ZIKAlliance

1. **Key-note lecture: Clinical outcome of congenital infections: lessons from a congenital CMV cohort (15')**
   Speaker: Ann Vossen, Leiden University Medical Center - ZIKAlliance

Q & A (5')

2. **Do we still need the cohorts? (15')**
   Speaker: Thomas Jaenisch, University of Heidelberg – ZIKAlliance

Q & A (5')

3. **Conflicting evidence on the risk estimate of complications after ZIKV infection in pregnancy – why is the Rio estimate so high? (15')**
   Speaker: Patricia Brasil, Oswaldo Cruz Foundation – ZIKAlliance

Q & A (5')

4. **Updated data from the pregnant women cohort in the French Territories in the Americas (15')**
   Speaker: Bruno Hoen, Inserm – ZIKAlliance

Q & A (5')

5. **Limitations of case and exposure definition in epidemiological studies of ZIKV infection (10')**
   Speaker: Ricardo Ximenes, University of Pernambuco – ZikaPLAN

Q & A (5')

6. **First results from ZIKAlliance – Ultrasound monitoring of at risk pregnancies – how many Ultrasounds & when were they carried out (5')**
   Speaker: Julius Schretzmann, Heidelberg University Hospital – ZIKAlliance*

Q & A (5')

7. **Zika and other arbovirosis in times of humanitarian crisis: The case of Venezuela**
   Speaker: Adriana Tami, University Medical Centre Groningen – ZIKAlliance

Q & A (5')

Total: 115' (1h55)

10.30 – 11.00 ***Coffee Break***

11.00 – 12.30 **Session 5: Lessons from imported cases**

Chair: Denis Malvy, Central Hospital of the University of Bordeaux - ZIKAlliance

Co-chair: Antoni Soriano-Arandes, Barcelona University Hospital Vall d'Hebron – ZIKAction

1. **Key-note lecture: ZIKA imported cases (15')**
   Speaker: Denis Malvy, Central Hospital of the University of Bordeaux – ZIKAlliance

Q & A (5')

2. **Zika & pregnant women (10')**
   Speakers: Carlota Rodó, Barcelona University Hospital Vall d'Hebron - ZIKAction

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3. Zika & microbiologic diagnostic challenges (10')
Speakers: Elena Sulleiro Igual & Ariadna Rando, Barcelona University Hospital Vall d'Hebron - ZIKAction*

4. Serological response and clinical outcomes of children exposed to Zika virus during gestation: preliminary results of a prospective paediatric cohort study in a non-endemic country (10')
Speakers: Antoni Soriano-Arandes, Barcelona University Hospital Vall d'Hebron – ZIKAction, Ana Alarcón-Allen, Sant Joan de Déu University Hospital - ZIKAction

5. Zika & adult travellers: Travel-associated risk for local transmission (10')
Speakers: Diana Pou, Barcelona University Hospital Vall d'Hebron - ZIKAction

Q & A (5')

6. Zika virus incidence and diagnostic tools accuracy in French international travelers visiting Latin America and the Caribbean during the summer 2016 -The ZIKAMERICA cohort study (10')
Speaker: Thierry Pistone, Bordeaux University Hospital – Inserm - ZIKAlliance

7. What did we learn from European diagnostic labs (10')
Speakers: Marion Koopmans, Erasmus Medical Centre - ZIKAlliance

Q & A (5')

Total: 90' (1h30)

12.30 – 14.00 ***Lunch break/poster session***

14.00 – 15.45 **Session 6: Social & ethical implications of Zika**
Chairs: Nisia Verônica Trindade Lima, Oswaldo Cruz Foundation & Jocelyn Raude, Inserm - ZIKAlliance
Co-chair: George Haringuizen, Dutch National Institute for Public Health & the Environment - COMPARE

1. <u>Key-note lecture:</u> Zika Outbreak in Brazil: Challenges for Science, Public Health & Society (10')
Speaker: Nisia Verônica Trindade Lima, Oswaldo Cruz Foundation - ZIKAlliance

The sharing of pathogen genetic resources under the Nagoya Protocol: What are the ethical implications for international research on the ZIKAV? (10')
Speaker: George Haringuizen, Dutch National Institute for Public Health & the Environment - COMPARE

3. Narratives on Zika epidemic in Brazil: Challenges to science, politics & society (10')
Speaker: Gustavo Matta, Oswaldo Cruz Foundation - ZIKAlliance

4. The construction of the social representation of a health threat: Zika virus in the French press (10')
Speaker: Sylvain Délouvée, Rennes University - ZIKAlliance

5. Repercussions of Zika Virus Epidemic on the National Health System in Brazil and households (10')
Speaker: Claudia Pereira, Oswaldo Cruz Foundation - ZIKAlliance

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6. Zika and the state´s policy trail: a document review on the Ministry of Health´s responses (10')
Speaker: Carolina de Oliveira Nogueira et al.*, Oswaldo Cruz Foundation – ZIKAlliance*

7. Health promotion in the context of Zika epidemic: actors & scenarios in decision-making processes (10')
Speaker: Paulo Peiter, Oswaldo Cruz Foundation - ZIKAlliance

8. Production & circulation of knowledge about Zika: from scientists to social media users (10')
Speaker: Elaine Rabello, Oswaldo Cruz Foundation - ZIKAlliance

9. The cognitive & emotional representations of Zika & other mosquito-borne diseases in French Guiana (10')
Speaker: Jocelyn Raude et al.*, Inserm – ZIKAlliance*

Q & A (15')

Total: 105' (1h45)

15.45 –16.15 ***Coffee Break***

16.15 – 18.05 **Session 7: Other important questions + "late birds" presentations**
Chair: Patricia Sequeira, Oswaldo Cruz Foundation- ZIKAction
Co-chair: Ilana Lowy, Oswaldo Cruz Foundation – ZIKAlliance

1. Sexual transmission- interdisciplinary sub-session
***Key-note lecture:* Sexual transmission of Zika virus: the current state of affairs (15')**
Speaker: Ralph Huits, Antwerp Institute of Tropical Medicine - ZikaPLAN

The gender gap in the ZIKV infection: A social & behavioral perspective (10')
Speaker: Anna Friedler, Inserm – ZIKAlliance*

Zika virus infects the human testis and germline (10')
Speaker: Nathalie Dejucq-Rainsford, Inserm – ZIKAlliance

Origin of Zika virus in semen (10')
Speaker: Laurent Houzet, Inserm - ZIKAlliance*

Potential effect of Zika virus infection on human male fertility (5')
Speaker: Vivian Avelino Silva, University of Sao Paolo - ZIKAlliance

Q & A (5')

2. Other questions
Zika control in an ethical & legal context: the importance of fast data-sharing and the barriers hampering it (10')
Speaker: Carolina dos Santos Ribeiro, Dutch National Institute for Public Health & the Environment – COMPARE

A Zika virus research toolbox for ZIKAlliance & beyond (10')
Speaker: Martijn Van Hemert, Leiden University Medical Center – ZIKAlliance

Improving serological diagnosis of Zika virus to understand NeuroZika in Brazil (10')
Speaker: Raquel Medialdea Carrera, University of Liverpool - ZikaPLAN

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Zika virus infection perturbs osteoblast function (10')
Speaker: Noreen Mumtaz, Erasmus Medical Centre – ZIKAlliance*

Latest Zika virus outbreak: improve preparedness of disease control from the perspective of the "European Virus Archive goes Global (EVAg)" EU funded consortium (10')
Speaker: Jean-Louis Romette, Aix-Marseille University – EVAg

Q & A (5')

Total: 110' (1h50)

** Young researchers*

Wednesday June 6th, 2018

08.45 – 10.30 **Session 8: Basic research & antivirals**

Chair: Marc Lecuit, Inserm/Institut Pasteur - ZIKAlliance

Co-chair: Johan Neyts, University of Leuven – ZIKAlliance & ZikaPLAN

1. ZIKA virus replication in human placenta (10')

Speaker: Therese Couderc, Inserm – ZIKAlliance

Q & A (5')

2. Stress-induced unfolded protein response contributes to Zika virus-associated microcephaly (10')

Speaker: Laurent Nguyen, University of Liège – ZIKAlliance

Q & A (5')

3. Therapeutics for microcephaly (10')

Speaker: Ivan Gladwynng, University of Liège – ZIKAlliance*

Q & A (5')

4. ZIKV infection disregulates neurogenesis through the Notch pathway in human neural progenitor cells (10')

Speaker: Pauline Ferraris, IRD – ZIKAlliance*

Q & A (5')

5. Development of highly potent Zika/flavivirus inhibitors; lessons from HCV drug development (10')

Speaker: Johan Neyts, University of Leuven – ZIKAlliance & ZikaPLAN

Q & A (5')

6. Tomatidine, a novel antiviral compound against dengue & chikungunya virus" (10')

Speaker: Jolanda Smit, UMCG – ZIKAlliance

Q & A (5')

7. "ZIKV NS5: target-based drug development" (10')

Speaker: Bruno Canard, Aix-Marseille University – ZIKAlliance

Q & A (5')

Total: 105' (1h45)

10.30 – 11.00 ***Coffee Break***

11.00 – 12.30 **Session 9: Zika vaccine**

Chair: Frédéric Tangy, Pasteur Institute - ZIKAVAX & Nicola Viebig, European Vaccine Initiative - ZIKAVAX

1. <u>Key-note lecture:</u> Introduction to Zika vaccines & to the ZIKAVAX project (10')

Speaker: Frédéric Tangy, Pasteur Institute – ZIKAVAX

Q & A (5')

2. NHP Zika infection model (10')

Speaker: Romain Marlin, CEA – ZIKAVAX*

Q & A (5')

3. Talk 1: First generation MV-Zika vaccine – fast track clinical development (10')

Speaker: Sabrina Schrauf, Themis – ZIKAVAX

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	Q & A (5')
	4. A YFV-17D based chimeric live-attenuated Zika virus vaccine is highly effective against lethal Zika disease in a mouse model (10') *Speaker: Kai Dallmeier, University of Leuven – ZIKAlliance & ZikaPLAN*
	Q & A (5')
	5. Zika vaccine roadmap: obstacles & opportunities (10') *Speaker: Annelies Wilder-Smith, Umeå University – ZikaPLAN*
	Q & A (5')
	6. Phase II/III clinical trials: endpoints, clinical trial sites/(target) study population & ethical considerations (10') *Speaker: Thomas Jaenisch, University of Heidelberg – ZIKAlliance*
	Q & A (5')
	Total: 90' (1h30)
	END OF THE SYMPOSIUM
12.30 – 14.00	***Lunch break***

** Young researchers*

Surveillance, burden estimates, modelling

Posters

Remaining gaps regarding the knowledge of Zika virus animal hosts.

Gladys Gutiérrez-Bugallo [1], Luis Augusto Piedra [1], Magdalena Rodríguez [1], Juan Bisset [1], Ricardo Lourenco De Oliveira [2], Scott Weaver [3], Nikos Vasilakis [3], Anubis Vega Rua [*†] [4]

[1] Department of Vector Control, Center for Research, Diagnostic and Reference, Institute of Tropical Medicine Pedro Kourí, PAHO-WHO Collaborating Center for Dengue and its Vector – Cuba
[2] Fundação Oswaldo Cruz-Fiocruz, Instituto Oswaldo Cruz, Laboratório de Mosquitos Transmissores de Hematozoários, Rio de Janeiro – Brazil
[3] Department of Pathology, University of Texas Medical Branch, Galveston, Texas – United States
[4] Environment and Health Unit, Laboratory of Medical Entomology – Institut Pasteur de la Guadeloupe – France

Zika virus (ZIKV), discovered in the Zika Forest of Uganda in 1947, is a mosquito-borne flavivirus related to yellow fever, dengue and West Nile. Since its discovery and before 2007, ZIKV has been reported in sporadic outbreaks that took place mainly in Africa and Southeast Asia, where only mild clinical manifestations including fever, rash, arthralgia and conjunctivitis were reported in patients. For these reasons, little attention was given to this virus in the past. Despite the rising number of studies performed on ZIKV in the last three years, there were more than five decades of "silence" regarding the scientific production about this virus that led to important gaps in the knowledge of ZIKV, especially about the spectrum of species involved in the transmission cycles of this virus. Here, we present the results of a World Health Organization commissioned review regarding ZIKV potential animal host species. Seventy-nine animal species, at least, have been identified to be naturally or experimentally susceptible to ZIKV infection. They belong to 18 orders and four classes, which suggests that there is no clear association between ZIKV and a particular animal species. Among the species listed above, 63 have been identified as susceptible organisms to ZIKV in natural contexts, while *Macaca mulatta, Cercopithecus aethiops, Cercopithecus ascanius, Callithrix jacchus, Eidolon helvum and Capra aegagrus* have been recognized as susceptible hosts to ZIKV infection in both natural and experimental conditions. Unfortunately, few diagnostic methods used for ZIKV detection were able to determine viral load in vertebrate hosts. This gap need to be urgently filled by the scientific community to know if these potential animal host species can transmit a sufficient number of ZIKV particles to infect mosquitoes and transmit therefore the infection.

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Mobile Genomic Surveillance of Zika and Chikungunya Viruses in North Brazil

Marta Giovanetti [1], Felipe Naveca [2], Jaqueline Jesus [1], Felipe Iani [3], Ingra Claro [4], Paola Silveira [5], Joilson Xavier [1], Valdinete Nascimento [2], Victor Souza [2], Flavia Salles [4], Poliana Lemos [6], Gabriel Wallau [7], Rodrigo Carvalho [8], Marineide Da Silva [9], Joshua Quick [10], Jose Lourenço [11], Sarah Hill [11], Simon Dellicour [12], Julien Thezé [11], Renato Santana [5], Marcia Castilho [13], Catia Meneses [14], Marconi Gomes [14], Tiza Moreira [9], Alvaro Almeida-Couto [15], Emerson Castilho-Martins [16], Laura Cruz [17], Andre Abreu [17], Marcio Garcia [18], Ricardo Gadelha [18], Osnei Okumoto [19], Carlos Albuquerque [20], Marcio Nunes [6], Ester Sabino [4], Oliver Pybus [11], Nicholas Loman [10], Nuno Faria* [11], Luiz Alcantara †‡ [1]

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[2] Fundação Oswaldo Cruz – Amazonas, Manaus, Brazil
[3] LACEN-MG, Fundação Ezequiel Dias – Belo Horizonte, Minas Gerais, Brazil
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[9] Laboratório Central de Saúde Pública do Amazonas – Manaus, Amazonas, Brazil
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[14] Laboratório Central de Saúde Pública de Roraima – Boa Vista, Roraima, Brazil
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Zika virus (ZIKV) and Chikungunya virus (CHIKV) have been causing unprecedented epidemics. Brazilian northern region reported more than 25,000 of CHIKV suspected cases since 2014 and between 2016-2017, more than 2,327 of ZIKV. To gain insights into the timing,

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source, and likely route(s) of those viruses' introduction, we performed ZIKV and CHIKV complete genome sequencing from infected patients, as a part of the ZiBRA project framework (http://www.zibraproject.org).
We generated 56 ZIKV and 18 CHIKV genomes from Amazonas and Roraima states, respectively. Phylogenetic analysis showed that novel ZIKV sequences belongs to the Asian genotype and CHIKV sequences belongs to the ECSA genotype (bootstrap support = 99%, posterior support = 1.00). Genetic analysis suggests that ZIKV and CHIKV outbreaks most likely originated from transmission cycles not previously identified in North Brazil and not from a separated introduction into the Americas. Molecular dating analysis indicates that ZIKV outbreak was caused by a single founder strain that is estimated to have arrived in Manaus around February 2015. Surveillance and genetic data show that ZIKV moved among transmission zones in Manaus and geographical analysis further indicates that north part of Manaus has high transmission potential for ZIKV. In addition, phylogenetic reconstruction of ECSA genotype history in Brazil further suggests that this lineage was introduced into the Amazon region from the neighboring Bahia state, which experienced CHIKV epidemics during January–August 2015.
Our work illustrates that field near-real time genomics can augment traditional approaches to infectious disease surveillance and control. Estimated dates for international spread of ZIKV from Amazon region indicate the persistence of virus transmission in recipient regions. Also, unrecognized transmission of CHIKV ECSA genotype in North Brazil is unique in the Americas and the spread of this genotype in this region could be mediated by host immunity, vector suitability and human mobility.

Estimating the Risk of Microcephaly After Zika Infection in Pregnancy in Pernambuco, Brazil, via a Mathematical Model of Population Infection Dynamics

Neal Alexander *† [1], Wayner V. Souza [2], Laura C. Rodrigues [1], Luciana C. Albuquerque Bezerra [2], Paula Alexandra Dos Santos Oliveira [2], Cynthia Braga [2], Celina M. Turchi Martelli [2]

[1] London School of Hygiene Tropical Medicine – United Kingdom
[2] CPqAM/FIOCRUZ for the Microcephaly Epidemic Research Group – Recife, Brazil

Maternal infection with the Zika virus was determined to be the cause of an epidemic of congenital microcephaly, which started in Brazil in 2015. We use multiple data sources, linked with a compartmental mathematical model, to estimate the risk of microcephaly given Zika infection in pregnancy, in the state of Pernambuco in northeast Brazil. Between 1 August 2015 and 16 August 2016, 1,767 live births were recorded as potential microcephaly cases, of which 652 had head circumference below the 3rd percentile of the Intergrowth standard curve. Denominators, in terms of numbers of live births, were estimated from the official registry. The occurrence of microcephaly shows a symmetric peak in November 2015 followed by a slow tailing off. Seroprevalence after the peak of the epidemic, i.e. the risk of infection at any previous time, was estimated at 57%, corresponding to a value of approximately 1.5 for R0, the basic reproduction number. For the present purpose we estimate the risk of infection occurring only within a specific time window during pregnancy, in terms of gestational age. This risk will vary over time according to the state of the infection epidemic in the population. Estimation is done via a SEIR (Susceptible, Exposed, Infectious, Recovered) model, fitted with the deSolve package in R. This model can capture the main peak of infection but not the tailing off. We assume 4 days' duration of the latency ('E') stage,. Initially we assume constant biting rate of the *Aedes* mosquito vector. The data can be fitted by different combinations of a) the duration of the infectious ('I') stage and b) the time window, in terms of gestational age either side of the middle of the first trimester, during which an incident maternal Zika infection may induce foetal abnormalities. For a 6-week window, an infectious duration of 4 days fits the main peak and implies that, at the time of greatest force of infection, approximately 30% of pregnant women became infected in that 6-week window, and that in approximately 5% of such pregnancies the neonate was born with microcephaly.
This is much higher than the risk of microcephaly in all pregnancies, because the denominator is assumed only to be those pregnancies with an infection in the specified period. Likewise, a 2-week window fits the data with an infectious period of 6 days, implies a peak of 12% of women becoming infected during that window, and a 12.5% risk of microcephaly among such

*Speaker
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pregnancies. We will also present results with a model which allows vector biting to vary in accordance with data from the LIRAa public health system for *Aedes* surveillance and control.

Characterisation of Population Exposure (Seroprevalence) to Arboviruses after recent Outbreaks in Colombia: dengue, chikungunya and Zika

Zulma Cucunuba * [1], Pierre Nouvellet [2], Isabel Rodríguez-Barraquer [3], Jessica Vanhomwegen [4], Maria-Consuelo Miranda [5], Erwin Camacho Burgos [6], Andrea Trujillo Correa [7], Maria I. Estupinan [8], Anyela Lozano [8], Margarita Gelves [8], Victor-Mauricio Herrera [9], Rita Almanza [10], Jean-Claude Manuguerra [11], Maria-Gloria Basanez [1], Christl A. Donnely [1], Jorge Osorio [12], Luis Angel Villar [5], Neil M Ferguson† [1]

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The 2015-16 Zika (ZIKV) epidemics in Latin America took the world by surprise, much as did the chikungunya (CHIK) epidemics in 2013-14. Nevertheless, the extent of exposure among the population at risk is still uncertain. Based on probabilistic population sampling, between October and December 2016 we conducted household-based seroprevalence studies in four different cities in Colombia, South America (Cúcuta, Neiva, Sincelejo and a sector of the city of Medellín). Over 2400 participants were enrolled, provided a blood sample and answered a questionnaire assessing risk factors for exposure. Prior infection by dengue (DENV), ZIKV and CHIK was ascertained using a multiplex recombinant antigen-based microsphere immunoassay measuring IgG against the three viruses. We found large variation in seropositivity, suggesting large variation in the rate of infection between the four cities. For DENV, seropositivity varied from 48.9 (CI 44.8 - 53.0) to 88.9 % (CI 86.1 - 91.3); for CHIK, seropositivity varied from 7.1 % (CI 5.2 - 9.4) to 72.3 % (CI 68.7 - 75.8); while for ZIKV, seropositivity varied from 6.7% (CI 4.8-9.0%) to 65.9 (CI 62.0 - 69.6). The marked increase in DENV seropositivity with age confirmed the endemic nature of its transmission, whereas exposure to both ZIKV and CHIK appeared less related to age confirming their epidemic nature. Among the four cities, Medellin

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had the lowest seroprevalence for all the three viruses measured. Interestingly, history of DENV or ZIKV disease in the past was not associated with antibody presence against these viruses. In contrast, having been previously clinically diagnosed with CHIK was strongly associated with being seropositive to CHIK (OR 10.32; 95% CI 7.20 - 14.79) and 41.16% of individuals who tested positive for CHIK IgG reported presenting symptoms associated to this infection in the past. This study provides the first estimation of population attack rates and exposure levels to two emerging arboviral infections in Colombia, a Dengue endemic country, and provides key elements towards the general understanding of the transmission patterns of arboviral infections.

Global Birth Defects Surveillance Inventory and Tablet/Mobile App

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The Zika epidemic has brought the world's attention to the need for better congenital anomaly (birth defects) surveillance systems, especially in lower resource countries/regions. Birth defect surveillance systems are crucial to the understanding of congenital anomaly causation, prevention and forward healthcare planning. In relation to Zika, they are crucial to tracking the course of, and size of, the epidemic. The International Committee for Congenital Anomaly Surveillance Tools has been created, as part of Workpackage 8 of the EU-H2020 funded ZikaPLAN project, to

- create an inventory of existing reources and tools for birth defect surveillance (guidelines, software, organisations, training) which will soon be available on the Global Health Network website.
- develop an App to be used on tablet and mobile phone devices which will assist healthcare professionals participating in birth defect surveillance systems in the identification, description and coding of a wide range of externally visible birth defects. The App contains a variety of photographs, illustrations, videos and other educational materials.

The International Committee has members representing ICBDMSR, EUROCAT, ECLAMC, CDC and WHO, from 9 countries of Europe, USA, Latin America, Africa and Asia. The inventory includes resources from all organisations represented as well as other relevant resources. The App has derived material in particular from the existing WHO/ICBDSR/CDC Birth Defects Atlas and ECLAMC Atlas.
We will present visual images of the development of these tools, and invite participants to give feedback on the use of the App.

*Speaker

The burden of Zika virus in the Pacific area

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Zika virus (ZIKV) emerged for the first time out of Africa in the remote Island of Yap, Pacific, in 2007. It disappeared epidemiologically from the Pacific and re-emerged unexpectedly in French Polynesia on late 2013 causing a huge outbreak. This outbreak revealed for the first time the occurrence of severe complications of ZIKV infection in adults (Guillain-Barré syndrome), along with the potential for materno-fetal, sexual and post transfusion transmission. The origin of introduction of ZIKV is French Polynesia is unknown. The strain belonged to the Asian ZIKV lineage and was close to a strain previously isolated in Cambodia. The new clinical pattern of infections reported in French Polynesia was probably linked to mutations that occurred in the French Polynesia strain. From French Polynesia ZIKV spread in the Pacific. Its circulation was detected in Marshall Islands, Fiji, Federated States of Micronesia, New Caledonia, Samoa, Cook Islands, Easter Island, Solomon Islands, Vanuatu, American Samoa, Papua New Guinea, Palau and Tonga. However data from other Pacific Islands are lacking and the burden of the disease in the Pacific is probably underestimated.
Of note, even if accurate data are not available for all Pacific countries affected by ZIKV, the outbreak profile can be very different depending on the location (huge outbreak in French Polynesia compared to limited circulation in other Pacific countries). From the Pacific, probably French Polynesia, ZIKV spread to the Americas were it was first detected in 2015. Of interest, the chikungunya strain the emerged in the Americas in 2013 (St Martin Island) was also probably introduced from the Pacific. These data suggest that, for indeterminate reasons, tropical islands, especially Pacific islands are new hubs for emerging arboviruses.
These data also highlight the need for improving active vector-borne pathogens surveillance and research programs in remote areas, especially the Pacific.

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Surveillance, burden estimates, modelling

Oral presentations

Real-time assessment of healthcare requirements during the Zika virus epidemic in Martinique

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Introduction: The spread of Zika Virus (ZIKV) during the 2015-2016 epidemic has been associated with a surge in Guillain-Barre Syndrome (GBS), a usually rare autoimmune disease that can result in near-total paralysis. Given GBS severity, territories affected by ZIKV needed to plan healthcare resources to manage GBS patients. French authorities were confronted with these issues in December 2015, when a ZIKV epidemic started in Martinique, a French island in the Caribbean. What would be the expected number of GBS cases? How many GBS cases would require intensive care or mechanical ventilation during the epidemic?
Methods: To inform planning in Martinique, we analyzed in real time ZIKV surveillance and GBS data from Martinique with a modeling framework that captured ZIKV epidemic dynamics, the risk of GBS in ZIKV infected persons, and the clinical management of GBS cases; and compared our estimates with those from the 2013-2014 ZIKV epidemic in French Polynesia.

Results: The basic reproduction number was lower in Martinique (1.36; CI: 1.30-1.42) than in French Polynesia (1.61; CI: 1.53-1.69), resulting in a lower predicted attack rate of 48% (CI: 43%-53%). The risk of developing GBS following ZIKV infection was 1.58 per 10,000 ZIKV infections (CI: 1.04-2.22) and was lower than in in French Polynesia, although the difference was borderline significant. We were able to predict just few weeks into the epidemic that the total number of GBS cases in Martinique would be substantially lower than what suggested by naïve extrapolations from French Polynesia. We also correctly predicted that 8 intensive care beds and 7 ventilators would be sufficient to treat GBS cases.
Discussion: This study showcases the contribution of modelling to inform local healthcare planning during an outbreak. Timely studies that estimate the proportion of infected persons that seek care are needed to improve the predictive power of such approaches.

*Speaker

Seroprevalence of Zika, Dengue, and Chikungunya in a community based survey in Northwestern Burkina Faso

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The Seroprevalence of members of the Flavi- and Alphavirus families in rural regions of West Africa is largely unknown. Of special interest is the seroprevalence against Dengue and Zika viruses. Due close phylogenetic relationships and antigenic similarities, cross-reactive immune responses cause large uncertainties in the interpretation.
Here we report about seroprevalence and cross-reactivity of Zika, Dengue, and Chikungunya (Flavi- and Alphavirus family) in a community based study in a rural region of West Africa.

The study site is located in Nouna, Northwestern Burkina Faso. Samples were collected to investigate genotypic resistance against malaria parasites over time. The local partner is the Centre de Recherche en Santé de Nouna, Burkina Faso.

From 2009 till 2012, 561 blood samples from individuals have been taken in three rainy and three dry seasons. We ingestigated the antibody responses in 561 samples (screening Dengue ELISA), backed up by 104 randomly selected samples with PRNT.

When testing the samples with the Dengue Elisa about 60% of samples were tested positive (53%, 300 out of 561) or indeterminate (7%, 40 out of 561). In comparison, the Zika Elisa only revealed 9.4 % positive (6.4%, 34 out of 530) or indeterminate (3%, 19 out of 530) results. The results will also be presented stratified by age group and over time (2009-12). The age-specific seroprevalence rates show a steady increase and now signal of epidemic activity.

Around 39% of the 90 Dengue Elisa positive samples were tested positive by Dengue PRNT, highlighting the issues with specificity and potential cross-reactivity between the family members of Flaviviruses.
The results show a considerable seroprevalence against Dengue and Zika in a rural community of Northwestern Burkina Faso, which is surprising as does not conform with the features of urbanity, climate, and mobility that are typical for the dengue pandemic.

*Speaker

Evidence of Zika virus circulation in Mali

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After the threat of the Ebola outbreak in West Africa in 2014 that affected Mali, Malian and French researchers decided to make a health system to control emerging viral diseases. One part of the work was assessing the seroprevalence of Zika virus infection, which was considered as emergency by the WHO because of congenital malformations and neurological complications such as Guillain-Barré syndrome and microcephaly. The purpose of this work was to map the presence of Zika virus across the country. Bamako, Bandiagara, Bougouni, Diema, Kadiolo, Kita and Niono were the study sites with different echoclimatic settings. Thus, 793 sera from asymptomatic volunteers aged more 14 years were analyzed for the detection of IgG anti ZIKA by ELISA and virus seroneutralization test. The overall seroprevalence was 12.0%; it varied between 3.1% for Niono and 20.2% for Diéma. No statistical difference was detected between male and female (11.6 % versus 12.2 %, Pearson $p = 0.814$), except in Bougouni with the seroprevalence higher in male than in female (9/42 = 21.4% for male versus 6/85 = 7.1 % for female, $p= 0.018$). Total Zika virus seropositivity is increasing as the groups age increases (7.7% for 15-29 years, 12.8% for 30-44 years, 21.4% for 45-59 years, 22.6% for ≥60 years). These data showed the Zika virus circulation in Mali with high seroprevalence in some area. Keywords : Zika virus, IgG antibodies, seroprevalence, Mali.

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Seroprevalence of Zika Virus in Cameroon and Republic of the Congo

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Zika virus (ZIKV) was first isolated from a caged rhesus monkey in Uganda in 1947 but its epidemiology in Africa remains poorly characterised. Here, we provide some information regarding estimates of the current immunity rate and dynamics of infections in Cameroon and Republic of the Congo, based on seroprevalence studies performed in volunteer blood donors.
A total of 529 sera collected from blood donors in Republic of Congo between March-July 2011, and 1084 sera from 6 different regions of Cameroon collected between August-October 2015 was tested for the presence of IgG antibody to ZIKV. All sera were firstly tested with anti-NS1 ZIKV IgG ELISA kit (EUROIMMUN, L'ubeck, Germany). Subsequently, ELISA non-negative samples were subjected to a cytopathic effect-based virus neutralization test (CPE-based VNT). Samples with a threshold $\geq$40 were recorded as positive following recommendations of the French National Reference Centre for Arboviruses.

After VNT confirmation, ZIKV seropositivity was low in both countries, *ca.* 5% in Cameroon (ranging between 2% and 10%) and 2% in the Republic of Congo (1-5%). In Cameroon, seropositivity was associated with epidemiological markers compatible with a (peri-)sylvatic transmission cycle.
These results indicate that the populations of Cameroon and Republic of Congo are broadly immunologically na'ive against ZIKV. Therefore, a risk exist that ZIKV strains efficiently transmissible by peri-domestic mosquitoes could cause epidemics in these countries. To our knowledge this is the most recent report indicating ZIKV seropositivity in Cameroon and Republic of Congo.

*Speaker

Zika virus seroprevalence study in Bolivia

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Between December 2016 and April 2017, a ZIKV seroprevalence study was conducted in 814 asymptomatic Bolivian volunteer blood donors residing in five departments corresponding to various eco-environments and different entomological activities to estimate the future potential circulation of the virus. It was based on detection of IgG to ZIKV using NS1 ELISA screening, followed by a seroneutralisation test in case of positive or equivocal ELISA result. ZIKV circulation occurred in tropical areas (Beni: 39%; Santa Cruz de la Sierra: 21.5%) but not in highlands (_˜0% in Cochabamba, La Paz, Tarija). Cases were geo-localised in a wide range of urban areas in Santa Cruz and Trinidad. No differences in seroprevalence related to gender or age-groups could be identified. We conclude that further intense circulation in the Beni region is unlikely, but in Santa Cruz the seroprevalence was still limited and the density of *Aedes aegypti* populations makes possible the spreading of the disease. This result was used to set up a cohort study of ZIKV infection in pregnant women in Santa Cruz.

*Speaker

Zika seroprevalence study Suriname

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Here we present the results of a Zika virus (ZIKV) seroprevalence study conducted in Suriname. Serum samples were collected in January and February 2017, one year after the peak of reported cases of ZIKV in Suriname, in one urban area (the capital Paramaribo) and two rural areas in the rainforest. In Paramaribo, the recruited participants were patients who visited the emergency department of the Academic Hospital Paramaribo with all urgency codes except those who were triaged with code 1 (life threatening). In the rural rainforest areas, participants were recruited via a household survey in which one member of a household was asked to participate in this study.
In total, 769 participants were recruited and all samples were tested on a ZIKV virus neutralization assay and a commercial ZIKV IgG ELISA from Euroimmun. We also tested serum samples from inhabitants of Suriname collected in 2012, 2013 and 2014, so before the introduction of ZIKV in Suriname, all 44 samples tested negative for ZIKV neutralizing antibodies.
Preliminary testing showed a ZIKV neutralizing antibody seroprevalence of 30%, suggesting ZIKV could reoccur if conditions are favorable for spread. Further testing is ongoing, the final results will be presented.

*Speaker

Modelling & predicting Zika circulation in Latin America

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In 2013 Zika virus (ZIKV) was introduced into northeast Brazil and has since spread throughout the Latin America & the Caribbean (LAC) region, but cases have been steadily declining since 2017. Such declines are consistent with predictions from many early mathematical models that predicted a build-up of heard immunity and elimination of ZIKV for a number of years. Since then, new data and greater understanding of the transmission dynamics of ZIKV have enabled such models to be improved. Here we present the first model to predict spread of ZIKV in the LAC region that has been fit to the now abundant data across many countries in the region. We use this model to make predictions of ZIKV incidence in 2018 including uncertainty from 10-fold cross validation.

These models suggest limited transmission in 2015, was followed by wide-spread outbreaks in most cities in 2016 and 2017 before decline. We predict that the highest incidence in 2018 will be in Argentina, Colombia and some Brazilian States (Sao Paulo and Rio de Janeiro), but the estimated number of cases will be no more than a few hundred due to significant impact of heard immunity.

However, this model-predicted universal spread and exhaustion of the population contrasts with observed patterns of microcephaly, particularly in Brazil where the incidence of microcephaly in the northeast was considerably higher than in other parts of the country. Analyses from this dataset suggest transmission was heavily restricted to the Northeast of the country while areas in the south had highly focal but lower magnitude epidemics.
Ultimately only population-representative seroprevalence surveys coupled with modelling studies can reveal the likely future risk of ZIKV transmission. Examples of these are currently limited. In Salvador, Brazil such a study showed that ZIKV seroprevalence had marginally exceeded the threshold for heard immunity, but significant pockets of susceptibility remained even in this high transmission intensity setting. Determining the cause, frequency and geographic distribution of these susceptible pockets will be key in estimating the future risk and burden of ZIKV.

*Speaker

High seroprevalence against Zika virus in Salvador, north-eastern Brazil limits the potential for further outbreaks

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During 2015-2016 Brazil notified more *Zika virus* (ZIKV) cases than any other country, yet population exposure remains unknown. Serological studies on ZIKV are hampered by cross-reactive immune responses against heterologous viruses. We conducted serosurveys for ZIKV, *Dengue* (DENV) and *Chikungunya* (CHIKV) *virus* in 633 individuals prospectively sampled during 2015-2016, including microcephaly and non-microcephaly pregnancies, HIV-infected patients, tuberculosis patients, and university staff in Salvador, north-eastern Brazil using ELISAs and plaque-reduction neutralization tests. Sera sampled retrospectively during 2013-2015 from 277 HIV-infected patients were used to assess temporal spread of ZIKV. Individuals were georeferenced and sociodemographic indicators were compared between ZIKV-positive and -negative areas, and areas with and without microcephaly cases. Epidemiological key parameters were modelled in a Bayesian framework. ZIKV seroprevalence increased rapidly during 2015-2016, reaching 63.3% by 2016 (95% confidence interval (CI), 59.4-66.8%), comparable to DENV

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(75.7%; CI, 69.4-81.1%), and higher than CHIKV (7.4%; CI, 5.6-9.8%). Of 20 microcephaly pregnancies, 95.0% showed ZIKV-IgG antibodies, compared to 68.8% of 256 non-microcephaly pregnancies (p=0.017). Analyses of sociodemographic data revealed higher than average ZIKV prevalence in low socio-economic status (SES) areas. High seroprevalence, combined with case data dynamics allowed estimates of the basic reproduction number R0 of 2.1 (CI, 1.8-2.5) at outbreak onset and an effective reproductive number Reff

High baseline prevalence of microcephaly in Zika-epidemic and non-epidemic regions: data from sub-Saharan Africa, Asia, and the Antilles

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Background: In regions of sub-Saharan Africa and Asia that are suitable for Zika virus transmission, information on the baseline prevalence of microcephaly in live born infants is needed to inform local surveillance strategies. Equally, in regions recently experiencing Zika epidemics such as the Antilles (Guadeloupe, Martinique), an understanding of the baseline prevalence of microcephaly and small-for-gestational-age would allow for a better estimate of the Zika-attributable risk of birth defects following infection during pregnancy. **Methods:** In large urban maternities in Yaoundé (Cameroon), Abidjan (Ivory Coast), Colombo (Sri Lanka), and Guangzhou (China), head circumference data was collected consecutively in live born infants. In Guadeloupe and Martinique, data on live births from a retrospective cohort of Zika-negative pregnant women was analysed. Moderate and severe microcephaly were defined as head circumference between -2 and -3SD, and less than -3SD, respectively, and small for gestational age was defined as having a birth weight of -1.28 SD, according to the INTERGROWTH-21st growth standards for gestational age and sex. **Results:** Data from 16607 live births was analysed from sub-Saharan Africa and Asia, as well as from 392 pregnancies without Zika exposure from Guadeloupe and Martinique. The prevalence of moderate and severe microcephaly in sub-Saharan Africa and Asia ranged from 2.4% to 25.8%, and 0.2% to 9.0%, respectively. In Guadeloupe and Martinique, the prevalence of microcephaly and small for gestational age in live births of women not infected with Zika during pregnancy was similar to that in women infected with Zika during pregnancy. **Conclusions:** In Zika non-epidemic settings, a cut-off of -2SD for microcephaly will most likely lead to labelling large numbers of healthy babies as

*Speaker

having microcephaly, with important regional variations; this will limit the ability to detect incoming epidemics. In epidemic settings, the baseline prevalence of anthropometric abnormalities should be considered in order to estimate of the ZIKV-attributable risk of birth defects; this has important implications for prenatal counseling.

Endemicity and emergence of arboviruses in Colombia: Insight from population based serological studies

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For diseases like dengue (DENV) or Zika (ZIKV), where a large proportion of infections are asymptomatic, population-based serological studies remain the gold-standard to quantify transmission. Here, we present findings from our population based studies conducted in Colombia since 2014. These studies include cross-sectional sero-surveys conducted among over 3500 persons living in 5 cities in 2014 and 2016, and an ongoing longitudinal cohort in Piedecuesta, Santander that enrolled 2400 participants in 2015. Follow-up within the cohort study involves active fever surveillance (via a call center) as well as yearly visits in which a blood sample is collected. Serological testing is being performed using a multiplex recombinant antigen-based microsphere immunoassay that simultaneously quantifies IgG against multiple arboviruses. This assay was validated using over 400 well characterized samples from the cohort study and shown to have good sensitivity and specificity for CHIKV, DENV and ZIKV.

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Low Zika virus seroprevalence in Vientiane, Laos, 2003-2015

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The first evidence of *Zika virus* (ZIKV) circulation in humans in Southeast-Asia (SEA) was obtained in the 1950s through serosurveys. However, the first human laboratory confirmed case in Asia was only reported in 2010, from Cambodia. ZIKV has been presumed to be endemic in the region, with a low rate of human infections in SEA, but its actual epidemiological status remains uncertain due to the scarcity of available data. From 2016, subsequent to the large outbreaks in the Pacific and Latin America, several Asian countries started reporting increasing numbers of confirmed ZIKV cases, but no global epidemiological assessment is available to date. Here, with the aim of providing information on ZIKV circulation and population immunity, we conducted a seroprevalence study amongst blood donors in Vientiane, Laos. Sera from 359 asymptomatic consenting adult donors in 2003-2004 and 687 in 2015 were screened for anti-ZIKV IgG using NS1 ELISA assay (Euroimmun, Germany). Positive and equivocal samples were confirmed for anti-ZIKV neutralizing antibodies by Virus Neutralisation Tests (VNT).
Our findings suggest that ZIKV has been circulating in Vientiane over at least the last decade. ZIKV seroprevalence observed in the studied blood donors was low, 4.5% in 2003-2004 with an increase in 2015 to 9.9%, possibly reflecting the increase of ZIKV incident cases reported over this period. We did not observe any significant difference in seroprevalence according to gender. ZIKV transmission cycle remains poorly characterised.
With a low herd immunity in the Lao population, ZIKV represents a risk for future large scale outbreaks. Implementation of a nation-wide ZIKV surveillance network as well as epidemiological studies through the country are needed.

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Clinical biology

Posters

Early Prenatal Zika Virus Infection Is Associated With Persistent Abnormalities In Eyes, Testis, Bone And Brain Structure Of Adult Offspring

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Introduction: *Zika virus* (ZIKV) infection is a global health emergency associated with serious neurological complications, including microcephaly in babies born from infected mothers. Recently, World Health Organization adopted additional criteria to diagnose ZIKV infection such as eyesight or hearing impairment and limb deficiency in addition to cephalic perimeter measurement, suggesting that severe microcephaly is only the "tip of iceberg". Indeed, it is known that several infections during pregnancy may be related to development of several neuropsychiatric disorders secondary to changes in the neurodevelopmental process, suggesting a relationship between the immune system and the brain development. However, the long-term consequences on the offspring born from ZIKV-infected dams are still not elucidated.
Objectives: Our aim was to evaluate the effects of early ZIKV infection on the neurodevelopmental, ophthalmological, bone and testicular abnormalities of the offspring born from infected dams. Additionally, we also evaluated the potential role of antibody-dependent enhancement, by administration of anti-envelope pan-flavivirus antibody (4G2), in the exacerbation of those abnormalities.

Methods: C57BL/6 pregnant dams were inoculated with 1x106 PFU of a Brazilian ZIKV strain (HS-2015-BA-01) by intraperitoneal (i.p.) route on gestational day 5.5 in the presence or absence of previous immunity (4G2 administration 10ug/mouse before infection and every 48hours

*Speaker

after infection). Negative and positive dam controls were injected with PBS or polyinosinic-polycytidylic acid potassium salt (poly I:C) by i.p. route, respectively. Intraocular pressure (IOP)1, behavioral tests (social memory test, y-maze, open field test, sucrose preference test and others)2, osteogenesis3, morphological analysis in testis3 and magnetic resonance imaging (MRI)3 started at four1, eight2 and twelve-week3 age of offspring, respectively.

Results: Reduced numbers of retinal ganglionar cells and increased apoptosis of those cells by caspase-3 immunostaining was detected in the offspring of ZIKV, 4G2-ZIKV and Poly I:C inoculated dams in comparison to PBS littermates. Those findings were associated with a marked increase in IOP levels, indicative of ophthalmological abnormalities especially in 4G2-ZIKV mice. Important morphological testis abnormalities, especially in the tubular compartment, such as seminiferous epithelium degeneration, sertolli cell deficiency, reduced tubular diameter and others were found in ZIKV-infected adult mice. Those findings were even more aggravated in 4G2-ZIKV group. Femur micro-computed tomography (microCT) analysis from the femur of ZIKV and 4G2-ZIKV infected mice revealed an osteopenic phenotype when compared to PBS mice, suggesting disruption in femur microarchitecture and loss of bone quality. Additionally, behavior analysis revealed slight alterations on basal locomotion, indicative of anxiety-like behavior, and a reduced glucose preference, suggesting anhedonia. Interestingly, magnetic resonance (MRI) revealed reduction in whole brain volume of all infected groups in comparison to PBS control.
Conclusions: Thus, our results reveal that early maternal ZIKV infection is associated to the development of important neurodevelopment outcomes of the offspring in adulthood. These results provide insights on clinical and neurodevelopmental consequences of early maternal ZIKV infection.

Clinical biology

Oral presentations

CD8 T cell immunity to Zika virus in humans is shaped by prior DENV exposure and associated with an IFN gamma/cytotoxic signature

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Several studies have characterized humoral immunity to Zika virus (ZIKV) in humans but little is known regarding the corresponding T cell responses to ZIKV. In this study we sought to investigate the ZIKV- specific T cell response in terms of kinetics and viral epitopes targeted and whether pre-existing dengue virus (DENV) T cell immunity can affect these responses. Our results shows that memory T cell responses elicited by prior infection with DENV infection or vaccination recognize ZIKV-derived peptides. The cross-reactive response is explained by the sequence similarity of the two viruses, as the ZIKV peptides recognized are either identical or highly conserved between DENV and ZIKV. In addition, DENV exposure also influences the timing and magnitude of the ZIKV-specific T cell response. In the acute phase of infection, ZIKV-reactive T cells are detected earlier and in greater magnitude than those in DENV pre-exposed patients. Conversely, while the frequency of ZIKV-reactive T cells continues to rise in the convalescent phase in DENV-naive donors, it declines in DENV pre-exposed donors. In addition, ZIKV-specific CD8 T cells form DENV pre-exposed donors selectively up-regulated granzyme B and PD1, as compared to DENV-naíve donors. These results overall suggest a more efficient control of ZIKV replication and/or clearance of ZIKV antigen in patients with previous DENV exposure. Finally, when we focused on the viral epitopes targeted by DENV naíve donors, ZIKV structural proteins (E, prM and C) are major targets of CD8 T cell responses, whereas DENV pre-exposed individuals skew the T cell epitopes primarily in nonstructural proteins.

*Speaker

Identification of a putative unique immunogenic Zika NS2b epitope for differential diagnosis and surveillance

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The current Zika virus (ZIKV) pandemic has been associated with unparalleled reports of neurological sequelae ranging from Guillain Barré syndrome to microcephaly, urging the development of vaccines and diagnostic tools to stop the infection spread. Major efforts have been made to develop effective vaccines, although no reliable diagnostic tools capable of differentiating ZIKV from other Flavivirus infections have been developed so far. The large clinical overlap and the well-known cross-reactivity between members of the Flavivirus family in serological assays remain a main challenge. We postulate that the identification of ZIKV-specific epitopes is crucial for the development of a specific serological assay. To this aim, the proteomes of 15 different ZIKV strains (retrieved from the NCBI database) were translated in overlapping linear 15-mer peptides as high-density peptide arrays. In order to identify antibody response profiles differentiating between ZIKV and DENV infections, the peptide arrays were tested against 84 clinically well-characterized patient and control serum samples. Among all peptides, a 15-mer sequence from NS2b was singled out as a promising candidate. The reactivity of the identified NS2b epitope was also evaluated by ELISA against a sample set with confirmed DENV or ZIKV infection only, from which 71% of subjects were shown to carry NS2b-specific antibodies. Nevertheless, the ELISA data also showed that the peptide was not capable to fully discriminate ZIKV from DENV infections. The quantification of the affinity of the identified epitope to the respective human IgG antibodies by microscale thermophoresis, a quantitative technique, will be discussed. Molecular dynamics simulations and cluster analysis were performed to assess the conformational entropy of the peptide free in solution. In addition, the 15 residues corresponding to the identified epitope were computationally grafted into a scaffold protein aiming to display the epitope in a stable NS2b native-like conformation. The presentation of the NS2b identified epitope via designed proteins in their native-like conformation is expected to amplify its ability to accurately distinguish Zika from other Flavivirus infections.

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The grim reality from a clinical researcher perspective

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Diagnostics remain a difficult theme in Zika research. In this presentation, we review the currently available tools, controversies and main limitations that are posed in clinical research for the diagnostic of Zika virus infection.

*Speaker

Models for biobanking for rapid assessment of diagnostics in emerging disease outbreaks

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Lack of access to well-characterised specimens has been identified as a major barrier to diagnostic test development. In an outbreak situation, diagnostic tests are needed for case detection, to determine the extent of the outbreak and to assess the impact of control strategies and interventions. Rapid and equitable access to well-characterised specimen obtained with ethical approval and informed consent can facilitate and accelerate test development and validation. Various models for how specimen biobanks can be set up with clearly defined guiding principles and rules for specimen acquisition, characterisation, storage and access by public and private sectors have been discussed but costs of setting up biobanks, willingness to share specimens, shipment of specimens across national borders, and sustainability of biobanks remain difficult issues to resolve. The affordability of and access to diagnostic tests for patients in the developing world adds to the complexity and fairness of biobanking for emerging infectious disease outbreaks.

*Speaker

Multiplex serology: new tools for complex problems

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Background: The highly similar clinical presentation and high proportion of asymptomatic cases in most arboviral diseases implies that it is virtually impossible to differentiate them without the use of laboratory methods. Additionally, the presence of several genetically close arboviruses within the same geographic regions, poses problems for the diagnostic specificity of serological assays due to the development of cross-reactive antibodies during the infections. To address the limitations of current serological tests we have developed a multiplex serological assay -entitled Arbo-MIA- for the differential detection of medically important arboviral diseases including Zika (ZIKV), Dengue (DENV), Japanese Encephalitis, West Nile encephalitis, Yellow Fever and Chikungunya (CHIKV) in a single test.
Methods: Large amounts of recombinant antigens from over 30 arboviruses have been produced through an easy, fast, and low-cost method and irreversibly conjugated to color-coded microbeads (MagPlex, Luminex corp.) using an optimized coupling protocol. The different antigen-coupled bead batches were combined to form a multiplex fluorescent microsphere immunoassay (MIA) allowing detection and differentiation of specific antibodies in patient sera, greatly reducing the required sample volume and turnaround time.

*Speaker

Results: The Arbo-MIA test has been implemented in collaborating institutes in several endemic countries, including French Polynesia, Bangladesh, Brazil and Colombia, and evaluated on well-characterized sample panels from over 500 confirmed arbovirus-infected cases. The results of this multicentric evaluation indicate high ($> 90\%$) clinical sensitivity and specificity for detection of IgG antibodies against ZIKV, DENV and CHIKV infection. The assay has been used to screen for antibodies in suspected ZIKV cases including children with birth defects, mothers who reported ZIKV-related symptoms during their pregnancy and Guillain-Barré syndrome cases. It has also been used to perform large-scale seroprevalence studies in human and animal populations from endemic areas.
Conclusion: The ArboMIA platform allows for rapid and simultaneous detection of antibodies to a wide range of arboviruses in biological fluids of infected patients, thereby providing a high throughput, cost-effective, and accurate tool for surveillance and diagnosis of Zika and other arboviral diseases.

Zika and Dengue antibody interactions: implications for diagnostics and vaccines

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Dengue and Zika viruses are simultaneously endemic in several regions. It is known that anti-flavivirus antibodies cross react. The effects of cross-reactions between Dengue and anti-Zika antibodies are not fully understood. It has been postulated that Zika and dengue cross-reactive antibodies can modify the clinical presentations, leading to protection or to more severe disease depending on the circumstances. We will present evidences supporting the effects of Zika and dengue interactions, its implications for dengue and Zika vaccines and the development of specific diagnostic tests.
Funding
CuraZika and ZikAlliance

*Speaker

Studying hypotheses for antibody enhancement in a cohort context

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Pre-existing antibodies triggered by dengue virus (DENV) infection can bind closely related DENV strains from other serotypes during secondary infection. These cross-reactive antibodies can fail to neutralize the virus and instead facilitate the entry of the virus into mononuclear phagocytic cells, resulting in more increased viremia and worsening of disease. This phenomenon is known as antibody dependent enhancement (ADE). Since ZIKV and DENV are closely related flaviviruses that co-circulate, ZIKV infected individuals are likely to have antibodies from previous DENV infection that could potentially result in ADE. To date, research suggests that there is in vitro evidence for enhancement of ZIKV infection, similar to what was observed for DENV, but not in animal models or humans. The focus here has been to study potential effects on disease severity, however other potential mechanisms of the influence of pre-existing cross-reactive flavivirus antibodies could be at the level of vertical transmission or virus tropism. Here I will discuss several hypotheses for the role of ADE of Zika virus infection, along with several challenges for studying this in the context of the ZIKAlliance cohort.

*Speaker

ZIKV cellular immune responses

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Zika (ZIKV) and dengue (DENV) viruses are closely related emerging flavivirus identical urban, mosquito-borne transmission. The emergence of ZIKV corresponded with enhanced disease severity and congenital syndrome, a phenotype characterized in part by severe neurological impairments. We will highlight the known immune-mediated responses that correlate with protective immunity to ZIKV and DENV infections, with a specific focus on Natural Killer (NK) cells and specific CD8+ T cells, as well as defense strategies utilized by flavivirus to counteract host antiviral immune responses. An approach based on an extensive immune profiling of ZIKV-infected patients will be discussed through a combination of mass-cytometry parameters for monocytes, dendritic cells, NK cells and lymphocytes, in addition to a functional, longitudinal, analysis with peripheral blood samples from ZIKV-infected Brazilian patients. This insight is critical to appropriately advance our understanding of the immune parameters that contribute not only to protective immunity, but also the symptomatic outcome during ZIKV infection.

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Zika virus diagnostics, the challenge

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Diagnostic methods are an essential component for outbreak research and response, and are needed to diagnose patients, rule out cases, track the spread of an outbreak, assess susceptibility of individuals or groups, investigate reservoirs, do surveillance and study sero-epidemiology. When dealing with an emerging disease outbreak, such diagnostic assays may not be available, or - when available - in limited supplies and with minimal knowledge on their performance. The Zika virus outbreak is no exception to this rule: while molecular detection assays have been considered a standard in the field of flavivirus diagnostics, their implementation in routine settings has been far from straightforward, due to variability of protocols, costs and availability of reagents, equipment and staff in the affected areas. Also, our limited understanding of kinetics of shedding of Zika virus, particularly in asymptomatic pregnant women and in (a)symptomatic infants, precludes clear conclusions at this stage on the use of PCR assays as a standalone diagnostic. Similarly, serology based assays are cumbersome in regions where persons have regular exposure to related flaviviruses, due to the presence of cross reactive antibodies, patterns of which may be different in different individuals. In addition to diagnostic application, for which assays with high discriminatory power are preferred, cross reactive antibody measurements need to be part of the investigational picture as they may be indicative of functional arms of the immune response, through mechanisms like antibody dependent cytotoxicity or antibody mediated enhancement. As long as major questions remain regarding the pathogenesis of Zika virus complications, it is important to keep an open eye on the pieces of information provided by the different measurements. In ZIKALLIANCE, this systems serology approach is one of the core aspects of the pathogenesis work.

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Vectors and vector control

Posters

The WIN Initiative: A Global Network to Combat Insecticide Resistance in Arbovirus Vectors

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Arbovirus transmitted by *Aedes* mosquitoes, such as dengue, Zika, chikungunya and yellow fever have been re-emerging all over the world. Vector control, mainly by the use of insecticides, play a key role in disease prevention but the use of the same chemicals for decades, together with the dissemination of vectors resulted in the global spread of insecticide resistance. A coordinated approach is imperative to detect and manage insecticide resistance and to deploy alternative strategies for vector control. Initiated with the support of the WHO Special Programme for Research and Training in Tropical Diseases (TDR) and the Department of Neglected Tropical Diseases (NTDs), the **Worldwide Insecticide resistance Network, WIN** (http://win-network.ird.fr/) brings together 19 internationally recognized institutions in vector research to track and combat insecticide resistance in mosquito vectors of arboviruses at a global scale. The missions of WIN are i) to establish a global resistance surveillance system for arbovirus vectors, ii) to fill **knowledge gaps** and identify **research priorities** on insecticide resistance, and iii) to assist WHO and national authorities in **decision-making** on insecticide resistance management and deployment of resistance-breaking tools. Since its creation in March 2016, the WIN has organized an international conference on vector resistance in Brazil and produced in-deep reviews to support the development of a global plan for insecticide resistance management in arbovirus vectors. The WIN is now entering into a new era by developing a membership organization open to new academia, public health agencies, international organizations, industries, NGOs, etc. to put insecticide resistance back in the vector control agenda.

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The role of field-collected Ae. japonicus in the potential transmission of ZIKV under constant and fluctuating temperature regime

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This study is investigating the susceptibility of field-collected *Ae. japonicus* to oral infection with two genotypes of ZIKV (Asian and African lineages). *Ae. japonicus* from two different geographic areas of Switzerland were collected as immature stages (eggs) and obtained adults exposed to rabbit blood spiked with either the Asian or the African strain. Engorged females were incubated under constant and realistic 'mid-summer' temperature regime (average, hot spells) in Central Europe. Several vector competence indices from survived females will be here investigated: infection (virus presence in the midgut) dissemination (virus presence in secondary mosquito tissues e.g. head, legs, thorax) and transmission (virus presence in the saliva) rates. Two major questions will be here addressed: i) Susceptibility of field-collected *Ae. japonicus* to oral infection with two genotypes of ZIKV (Asian and African); ii) Effect of temperature on ZIKV dissemination and transmission among *Ae. japonicus*

*Speaker

European Aedes caspius mosquitoes are experimentally unable to transmit Zika virus

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The Zika virus (ZIKV) was isolated for the first time in 1947 in the Zika forest (Uganda). It belongs to *Flavivirus* genus (*Flaviviridae* family). The virus is maintained in a zoonotic cycle between mosquitoes and non-humans primates and others mammals in Africa. It has been isolated from numerous African mosquito species of the genus *Aedes* sp., *Anopheles coustani*, *Culex perfuscus* and *Mansonia uniformis*. *Aedes aegypti* mosquito is the main vector to transmit ZIKV in urban populations. Infection in humans occurs mainly through the bite of infected female mosquitoes during blood feeding, although sexual and perinatal ZIKV transmission have been confirmed in humans. In addition, vertical and sexual transmission is possible in the vectors. *Aedes caspius* (Pallas, 1771) is a floodwater mosquito specie that tolerates different levels of salinity in larval breeding site. Consequently, it is widely distributed in coastland, irrigations canals, rice fields and swamps in Europe. It is anthropophilic and vector of several viruses, such as Chikungunya or Rift Valley fever phlebovirus. They are crepuscular feeders, most actively searching for a blood meal at dusk, but may bite during the day and night. In the present study, we evaluated the vector competence of one *Ae. caspius* mosquitoes population from El Prat de Llobregat (Barcelona, Spain) for two strains of ZIKV (Suriname and African MR766 strains). Females were artificially fed with blood containing ZIKV in BSL3 conditions. After a period of 7, 14 and 21 days post exposure to infectious blood, infection, disseminated infection and transmission rates and transmission efficiency were estimated. The virus was unable to induce a disseminated infection and transmission in *Ae. caspius*. Therefore, it is unlikely that *Ae. caspius* mosquitoes are involved in the transmission of ZIKV. Our results provide helpful information to health authorities in order to establish efficient surveillance and vector control programs for ZIKV.

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The Tiger Mosquito in Lebanon: An Expanding Public Health Threat

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Aedes albopictus is considered in many countries, a potential vector of several viruses such as Chikungunya (CHIKV), Dengue (DENV) and Zika virus (ZIKV). This mosquito was first observed in Lebanon in 2002. Since then, it has established in many geographic locations in the country and became a source of nuisance. In 2010, we showed that Lebanese strains of this mosquito are competent vectors for CHIKV and DENV. The purpose of the current study is to assess the geographic expansion of the tiger mosquito in Lebanon, its seasonal dynamic, its insecticide resistance profile and its vector competence toward ZIKV.
In order to map the current distribution of *Aedes albopictus*, BG-sentinel traps were placed in urban and rural habitats in almost 200 localities covering different bioclimatic zones: from arid to humid. Moreover, the pattern of its seasonal activity was assessed by determining the egg laying activity using ovitraps. The resistance profile of the Lebanese tiger mosquito against the most commonly used insecticides was determined using WHO standard bioassays: female specimens were exposed to Organochlorines (DDT 4%), Organophophates (Malathion 0.8%), Carbamates (Propoxur 0.1%), Pyrethroids (Permethrin 0.25% and Lamda cyhalothrin 0.03%). The presence of A302S mutation at the GABA gene and Ile1011Met mutation at the Kdr gene was investigated by RFLP and allele specific PCR respectively. The capacity of Lebanese strains to transmit ZIKV was assessed in BSL3 conditions by feeding starved females (F0) on infectious blood-meal containing rabbit erythrocytes and ZIKV suspension at a final Titer of 107.2 PFU/mL. Then batches of 12-30 mosquitoes were analyzed at 3, 7, 14, and 21 days post-infection (dpi) to estimate three parameters describing the vector competence: (i) the infection rate, the dissemination rate and transmission rate.

Collection data showed that *Aedes albopictus* is mainly spread in humid and subhumid areas on the western versant of the Mount Lebanon chain at different altitudes up to 1000m. The average number of laid eggs per trap and per week shows that the activity period of this mosquito extends from May till November and peaks in July and then in September. Insecticide resistance bioassays showed that the tested populations of the Lebanese tiger mosquito are susceptible to the used Pyrethroids and Carbamates insecticides but significantly resistant to DDT (80 % mortality) and more obviously to Malathion (20% mortality). On the genes

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level, 3% (n=34) and 2.5% (n=60) mutation rates were observed in the GABA and Kdr genes respectively. Preliminary results on ongoing vector competence revealed very high infection rate (94.11% at 14dpi) and dissemination rate (100 % at 21 dpi). The capacity of infected Tiger mosquito to secrete the virus is still ongoing.
The observed results highlight the need to establish a surveillance program to monitor and control the Tiger mosquito in Lebanon in order to reduce the risk of local spread of related arboviruses.

Vectors and vector control

Oral presentations

A new high-throughput tool to screen mosquito-borne viruses in Zika virus endemic/epidemic areas

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The primary vectors of Zika virus (ZIKV) mainly described in enzootic cycles in Africa are *Aedes* mosquitoes with reported viral isolations from different species. ZIKV was also isolated from other genera (*Anopheles*, *Mansonia*, *Eretmapodites* ...). In Asia, sylvan *Aedes* from the *niveus* group are suspected to be involved in monkey to monkey transmission. Urban *Aedes* mosquitoes are assumed to be the main ZIKV vectors in Latin America, especially the highly anthropophilic *Ae. aegypti*. The role of *Ae. albopictus* is unclear but its range of distribution is growing across several American countries. The virus was reported from *Ae. albopictus* in Central Africa during an urban ZIKV outbreak at Libreville. Besides, the role of an alternative urban species such as *Culex* has long been debated. The main objective of our work was to define the mosquito species involved in urban transmission of ZIKV in Latin America and the Caribbean and examine wild mosquitoes in rural environments with special emphasis on interface with forested areas. We chose sites in newly ZIKV-infected regions (Brazil, French Guiana, Guadeloupe, Surinam) and ZIKV-endemic countries (e.g. Africa with Senegal and Gabon).

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In those countries, adult mosquitoes have been collected, identified using morphological characters and dissected to separate abdomen from the remaining parts of body (RPB). Abdomens of a same species have been grouped by pools of 20-30 individuals in cryovials, and RPB have been stored individually. Total RNA have been extracted from each pool, reverse transcribed in cDNA and preamplified with primers targeted 58 viral species. Then those preamplified cDNA have been screened for the presence of 58 viral species by a new high-throughput method based on microfluidic realtime PCRs using the BioMark Dynamic arrays system. When pools of abdomens have been detected positive for viruses, the RPB (head/thorax) of individual mosquitoes composing each pool have been homogenized in medium, one aliquot was conserved for virus isolation, and one aliquot was used for RNA extraction and confirmation of the viral species by classical RT-realtime PCR.

Among the 10,000 mosquitoes collected in epidemic areas (*Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus*), we have detected ZIK, Una, Nepuyo, Chikungunya, West Nile and Trivittatus viruses. Isolation and sequencing of those viruses are under progress.

Regarding the mosquitoes collected at the interface with forested areas, in newly ZIKV-infected region such as French Guiana, no virus was detected from 1,000 mosquitoes collected. Nevertheless, more than 20,000 mosquitoes from Brazil (newly infected country) and more than 10,000 mosquitoes from Sénégal and Gabon (endemic country) are currently under screening.
This fast and low-cost method allows comprehensive testing of mosquito-borne viruses and can be customized to fit regional demands or to accommodate new or emerging pathogens. It represents a major improvement for surveillance and future epidemiological studies.

Worldwide survey of Aedes aegypti susceptibility to multiple strains of Zika virus

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Zika virus (ZIKV) is a mosquito-borne flavivirus mainly transmitted among humans through the bite of infected *Aedes aegypti*. After it was first isolated in Uganda in 1947, ZIKV was shown to circulate in enzootic sylvatic cycles in Africa and Asia but human infections remained sporadic for half a century. The first reported human epidemic caused by ZIKV occurred in 2007 on the Pacific island of Yap in the Federated States of Micronesia. Subsequently, larger ZIKV outbreaks were recorded in French Polynesia and other Southern Pacific islands in 2013-2014. In 2015, ZIKV reached Brazil from where it rapidly spread across South and Central America, infecting millions of people. The emergence of ZIKV caused significant public health concern because of the associated birth defects and neurological complications that have been observed since 2013. Until now, the factors that have fueled the explosiveness and magnitude of ZIKV emergence in the Pacific and the Americas are still largely unknown. Another unresolved question is the lack of major human epidemic of ZIKV in Africa despite seemingly favorable conditions. In order to evaluate the potential role of vector population diversity in the recent patterns of ZIKV emergence, we conducted the first worldwide survey of *Aedes aegypti* susceptibility for ZIKV in natural populations. We established dose-response curves for 8 field-derived mosquito populations spanning the entire geographical distribution of the species, following experimental

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exposure to 6 ZIKV strains representing the current extent of viral genetic diversity. Our data show that susceptibility to ZIKV infection varies substantially across mosquito populations, ZIKV strains, and their specific pairings. Most importantly, our results reveal that African *Ae. aegypti* are significantly less susceptible than non-African *Ae. aegypti* across all ZIKV strains tested. Thus, low susceptibility of vector populations may have contributed to prevent large-scale human transmission of ZIKV in Africa.

The Infravec2 Infrastructure Project: Providing Vector Researchers with No-cost Resources, Services and Facility Access

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The Infravec2 infrastructure project provides insect vector resources and facility access to researchers worldwide at no cost. The project is funded by the European Commission Horizon 2020 Research Infrastructure Program (INFRAIA). The 24 Infravec2 partners operate major European biosecure insectaries for experimental infection and containment of insect vectors and other key insect vector technology platforms including front-line field sites in Africa, the Pacific, and the Americas. Researchers can shop online and request resources from an extensive product catalog (www.infravec2.eu). Infravec2 is also developing innovative new research tools, providing training courses, and is networking the community with activities such as common experimental standards and protocols to obtain reproducible vector infection results across different facilities. Infravec2 is a source of EU research support for vector researchers. The Infravec2 goal is to accelerate European innovation in basic and translational insect vector biology, and to consolidate a high-quality insect vector infrastructure with long-term perspectives for improving global public health.

*Speaker

Identifying genomic changes associated with insecticide resistance in the dengue mosquito Aedes aegypti by next-generation sequencing

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Insecticide resistance threatens the control of mosquitoes transmitting arboviral diseases worldwide. Although alternative vector control tools are emerging, their global implementation will require some time and managing resistance to the few available chemical insecticides is crucial for sustaining vector control efforts for the next decades. In mosquitoes, insecticide resistance is mainly the consequence of modifications of the proteins targeted by insecticides (target-site mutations) and the biodegradation of insecticides by detoxification enzymes (metabolic resistance). Target-site mutations are relatively well characterized and easy to monitor using PCR-based diagnostic tools, while the genetic factors controlling metabolic resistance are far less understood impeding their monitoring in natural mosquito populations. In this context, we combined experimental evolution, quantitative genetics and next-generation sequencing approaches to identify novel genetic markers of insecticide resistance in the dengue mosquito *Aedes aegypti*. These studies revealed that copy number variations (CNV) and non-synonymous polymorphism variations affecting particular detoxification genes are strongly associated with resistance to different insecticides. This study paves the way to the development of novel diagnostic tools allowing to track the whole spectrum of insecticide resistance mechanisms concomitantly in natural populations in order to improve resistance management strategies.

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Potential of Aedes albopictus as a bridge vector at urban-forest interface in Brazil

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Being native from Asia, the tiger mosquito *Aedes albopictus* arrived to Brazil in the 80's being nowadays established in 60% of brazilian cities. This species represents a major concern for the transmission of epidemic arboviruses (dengue, chikungunya, Zika) but also a potential threat for the emergence of zoonotic diseases due to its presence in urban/forest interfaces, its opportunistic feeding behavior and its vector competence to transmit numerous viruses. Thus, *Ae. albopictus* might potentially participate as a bridge vector for the transfer to urban environments of numerous zoonotic arboviruses that are circulating on Brazilian forests.To test this hypothesis, we investigated the colonization, dispersion and the host feeding patterns of *Ae. albopictus* in the urban/forest interface. We studied several forest ecosystems of Brazil: Amazon forest in Manaus (Adolpho Ducke forest reserve), Atlantic forest in Rio de Janeiro (Pedra Branca forest) and Cerrado forest in Goîania (Hill Monkeys forest).
We studied the colonization of this species in the forest environment monitoring egg presence with ovitraps and the dispersion with BG-Sentinel traps located at a gradient from the edge to up to 900 meters inside the forest. Eggs and adult mosquitoes were identified and counted. Blood engorged females were analyzed to determine blood-meal hosts, and pools of females were tested using molecular tools caracterizing arboviruses presence, with aparticular focus on Zika and Yellow fever. Statisitical analysis (Generalized linear models) were perfomed to characterise colonization and dispersal patterns. Six additional urban/forest interfaces were investigated using a simplified protocol Salvador-BA, Serra-ES, Domingos Martins-ES, Simonésia-ES Belo Horizonte-MG, Casimiro de Abreu-RJ, Marica-RJ.

We observed that the forest colonization by *Ae. albopictus* decreases with the distance to the edge of the forest being detected until 300 meters inside the three forest areas. This results, together with other researches, confirmed that *Ae. albopictus* populations may decline as it

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penetrates into the forest. The dispersal of *Ae. albopictus* on the forests also decrease with the distance to the edge of the forest until 400 m in Adolph Ducke and Hill Monkeys. However, in Pedra Branca *Ae. albopictus* females abundance increase up to 200m and decrease up to 400m. Over all three sites, 66 *Ae. albopictus* blood engorged females were collected and analyzed revealing that host feeding patterns are clearly opportunistic, with a mark preference for humans and domestic mammals despite a contact with wildlife. Even if all *Ae. albopictus* tested for the presence of viruses were negative, these results confirmed a contact between *Ae. albopictus* and wildlife and with potential reservoirs of enzootic viruses. The presence of *Ae. albopictus* in the 6 additional forested sites indicated a general similar trends generalizable to a large area in Brazil.

Globally our results confirm and estimates the potential role of *Ae. albopictus* to act as a bridge vector of zoonotic diseases at the forest/urban interfaces in Brazil. This work opens a research area in which further investigations may assess the potential spill-over risk of zoonotic disease from forested to urban areas with the the aim to mitigate potential future viral emergences.

Vector competence to Zika of mosquitoes from Pacific region

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Zika virus (ZIKV) re-emerged in French Polynesia and then subsequently spread to the entire Pacific region (including New Caledonia in 2014, Fiji, Vanuatu and Samoa in 2015 and then American Samoa and Tonga in 2016).
In the Pacific region, little is known about the capacity of local mosquitoes to transmit ZIKV and sustain an outbreak. Along with *Aedes aegypti* the major vector, other *Aedes* species as *Aedes polynesiensis* or *Aedes albopictus* have been suspected to be involved in ZIKV transmission. In this study, we analyzed the vector competence of *Aedes* species from New Caledonia, Wallis and Futuna, Samoa and French Polynesia for an Asian lineage of ZIKV which circulated in the Pacific region during the 2013-2015 outbreak.

Both *Ae. aegypti* and *Ae. polynesiensis* were able to transmit the virus with an extrinsic incubation period ranging from 9 to 21 days, but with low transmission rates (about 20 %). These results suggest the implication of other factors in the outbreaks that occurred in the Pacific region, notably the large naïve human population for ZIKV and the high density of *Aedes spp* mosquitoes.

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Global analysis of the virome in Aedes aegypti mosquitoes: arthropod borne-viruses & beyond

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Insect borne diseases are accountable for approximately 750 thousand deaths per year around the globe. Mosquitoes are the major vectors for arthropod borne-viruses (arboviruses) of great public health importance. Recent outbreaks of Zika, Dengue Fever, Chikungunya, and Yellow Fever raised major concerns on the fast spread of these diseases. Identifying circulating viruses among mosquito populations is of key importance to predict outbreaks and guide public policies to avoid uncontrolled population health impact. Metagenomics is a powerful tool to investigate the collection of viruses in mosquito populations, including arboviruses and other components of the insect virome that could affect vector competence. Our group is focused on the identification of circulating viruses using high-throughput sequencing of small RNAs from insects. This strategy has important advantages such as that viral sequences are naturally enriched in the small RNA fraction of insects. Moreover, features like size distribution and nucleotide enrichment allow the identification of viral sequences independently of homology searches against reference databases. In this talk, I will present our most recent data on the analysis of the global virome of *Aedes* mosquitoes. Our preliminary results include the analysis of the virome in *Aedes* mosquitoes collected in 4 different countries and 3 continents on the scope of the Zikalliance partnership.

*Speaker

Optimization of odour-baited trapping systems for the surveillance & control of Aedes aegypti in Paramaribo, Suriname

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Our aim was to test odour-baited traps as an alternative method for mosquito surveillance and control. Blends of synthetic chemical attractants, derived from human skin emanations, can be used to attract host-seeking female mosquitoes. These blends gave rise to the development of odour-baited traps for mass mosquito trapping and control. Although tested to reduce malaria in Africa, these odour-baited traps might also offer a solution to diseases like dengue, chikungunya, and Zika, which are amongst others prevalent South American countries, including Suriname. For our study, we ran three Latin-square trials of BG traps of different design and in different configurations (with/without odour bait and with/without CO2) across eight different locations in urban Paramaribo. This study demonstrates the effectiveness of the BG-Sentinel when baited with a combination of CO2 and the MB5 blend for trapping *Ae. aegypti* females, and surprisingly also males. The MB5 blend outperformed the commercially available BG-Lure in the BG-Sentinel. Although to a lesser extent than the latter trap type, the BG-Bowl also attracted *Ae. aegypti* when baited with either the MB5 blend or the BG-Lure. Moreover, the results show that CO2, in our case provided by sugar-yeast fermentation, is an indispensable component of the attractive blend. The next step would be to focus on research that makes these traps feasible for usage in mass-trapping systems by searching for CO2 alternatives and evaluating the effect of trap placement and coverage.

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Cohort studies

Posters

A Multiplex qRT-PCR (ZIKV/CHIKV/DENV 1-4) assay : A reliable tool to improve cohort studies

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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. Real-time RT-PCR (qRT-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, a real time RT-PCR assay to detect specifically Zika virus was developed in our lab. Sequences retrieved from the Genbank database were used to designed primers and probe. We designed primers and probe specifics to NS5 protein. Then, we optimized a rapid RT-PCR in one step, using a reverse –transcriptase (RT) reaction to convert RNA into complementary DNA (cDna) in one step before PCR for the amplification of specific target. We checked the test's specificity toward other viruses and there was no cross reaction detected with CHIKV , DENV serotypes, West Nile virus, Yellow fever virus. 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 viruses are endemic in the same geographic regions and cause similar symptoms, the identification of the etiological agents is only possible in laboratory testing. That is the reason why we added to the ZIKV simplex test, a CHIKV and DENV (DEN-1, DEN-2, DEN-3, DEN-4) qRT-PCR detection in order to have a multiplex real time PCR assay. The assay also includes a G6PDH detection as a retro transcription control, and negative controls. In conclusion, the assay is rapid sensitive and specific to detect ZIKV, CHIKV and DENV(1-4) specific genomic RNA in serum and clinical samples for diagnosis purposes.

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Development of monoclonal antibodies against Zika's antigens

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In the frame of the workpackage 3 of ZIKAlliance project (toolbox sub task) several strategies were carried out to develop and select monoclonal antibodies (Mabs) against Zika's antigens. Mice and rats were immunized both with inactivated viruses and recombinant proteins, prME, NS1-6xHis and DIIIE-6xHis (provided by B. Coutard, AFMB, AMU, Zikalliance partner n○ 17). For NS1, two cell fusions were done using splenocytes from mice immunized with NS1-6xHis and 2400 hybridomas were obtained. Fifty seven were initially identified as positive on the primary screenings using coated NS1-6xHis recombinant protein. After all the complete screening procedure and the assessment of the growing capability of the clones, 18 hybridomas were selected and cloned produced according to our procedures. For all these Mabs, the ELISA signals (OD 450 nm) using coated NS1-6xHis, ranged between 2.5 and 4. Using the Biacore system (GE Healthcare), 13 Mabs showed high reactivity with NS1-6xHis in solution and 4 of them gave none or a very low resonance signal. It can be stressed that these 4 Mabs could be sensitive to the conformation of the antigen. The binding of the antibodies to others His tagged proteins was assessed and no cross reaction was observed with this tag. Fifteen MAbs showed positive binding to NS1 in Western blot. All the Mabs combinations were assessed in sandwich immunoassay formatunsing two concentrations of NS1, 50 and 500 ng/mL. On 324 antibody pairs, about 100 showed a high sensitivity (OD450nm $>$ 2.5) for the lower concentration of NS1 (50 ng/mL). For prME, three cell fusions were carried out using rat (1) and mice (2) immunized with cell lysis supernatants. They led to the growing of more than 3000 hybridomas. In the primary screenings, positive signals in ELISA were obtained using the coated immunogen. However, only low specific signals were observed in the secondary screenings using infectious viruses. Despite the immune response of the animals and the high number of hybridomas obtained, to date, no monoclonal antibody reacting with the infectious virus was selected using this strategy. Considering these first results, new immunizations were initiated with recombinant DIIIE-6xHis, leading to the selection of ten monoclonal antibodies against DIIIE, their characterization is ongoing. All these antibodies are available for all the interested teams of the ZIKAlliance consortium.

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The domain III of ZIKV-envelope protein is a specific target of ZIKV-infected patients' IgG antibodies.

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Zika virus (ZIKV) belongs to *Flavivirus* genus such as Dengue virus (DENV) and both co-circulate in several countries. ZIKV association to Guillain-Barré syndrome and severe outcomes during pregnancy, including microcephaly development in fetuses and neonates were highlighted during the America outbreak of 2015. Uncommonly for flaviviruses which are arthropod-borne virus (arbovirus), ZIKV can be sexually transmitted and infective ZIKV particles have been found in breastmilk with equivocal results of neonate infection and perinatal transmission. Flaviviruses are phylogenetically very close to each other and the induced antibodies present high cross-reactivity to their antigens and lead to Antibody Dependent Enhancement (ADE), increasing viremia. This cross-reactivity poses a problem to the development of a target molecule for reliable sero-diagnosis and for vaccine basis design. The domain III of the Envelop protein (EDIII) of many flaviviruses is known to induce virus specific antibodies. In this context, we studied a ZIKV-EDIII recombinant protein and described its recognition by a cohort of ZIKV positive, Flaviviruses positive but not ZIKV and negative sera. The aim of this study is to develop a specific and rapid diagnostic tool. In our assay, this antigen is recognized with 90% of specificity and 92% of sensitivity that make it a good target-candidate for serologic diagnosis. Its specificity could also lead to a good vaccine basis.

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Neurodevelopmental delays arising from in utero exposure to Zika virus in Salvador, Brazil

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Since 2015, Brazil has experienced an unprecedented Zika virus outbreak. A devastating consequence of this viral infection is congenital Zika infection (CZI), which is transmitted from pregnant women to newborns. Most descriptions and publications regarding CZI focus on the clinical presentation of newborns and infants with microcephaly. Scarce information is available concerning children without microcephaly born from infected mothers.
During 2016, in the city of Salvador (Bahia, Brazil), a cross-sectional study enrolled 147 pregnant women who reported an exanthematous disease during pregnancy. Of these, 101 (68.7%) presented anti-Zika IgG antibodies at the time of delivery. A total of 25 (17%) newborns were diagnosed with microcephaly, while 122 (83%) were classified as newborns without microcephaly, of these, 91 had positive Zika serology or Zika RT-PCR.
In June 2017, we began a prospective follow-up of these infants without microcephaly exposed to Zika Virus *in utero* by evaluating neurodevelopment delays, performing neurological examinations and applying the Bayley Scales of Infant Development III (BSID-III), Mental Development Index (MDI) and Bayley-III cognitive and language scales. Auditory evaluations were performed by Otoacustic emissions (OAE) and Brainstem Auditory Evoked Potential (BAEP). To date we have evaluated 26 infants, mean age 1.7 years. Of these, 53.8% are male and 61% were delivered by C-section. Anti-Zika IgG serology was positive in 77% and five (19.2%) presented positivity for Zika by PCR on urine samples within 24h of birth. Based on head circumference (HC) at time of birth, all were classified as normal by the Intergrowth scale and currently fall within normal HC percentiles. Cognitive delay was identified in 8 (31%) infants, language delay in 11 (42.3%) and motor delay in three (11.5%). Our preliminary results indicate that *in utero* exposure to Zika virus could be associated with neurodevelopmental delay, even in children born without microcephaly at birth. Currently, only microcephalic infants are referred to specialized care, while normocephalic children are maintained in primary health care. We believe that all newborns exposed to Zika *in utero* should be referred to specialized centers for the early detection of neurodevelopmental delays and timely intervention.

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Investigating Vertical Transmission of Chikungunya, Dengue and Zika Virus Infection: a Prospective Observational Cohort Study of Pregnant Women and Infants in Jamaica

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Background: Dengue, Chikungunya and Zika outbreaks have occurred in Jamaica in recent years, where all three viruses now circulate endemically. The peak of the Zika outbreak in Jamaica was in June 2016. While several pregnancy-related complications, including the fetal brain disruption sequence, have been associated with Zika, many knowledge gaps remain. These include the rate and risks of vertical transmission, pregnancy outcomes and the natural history of congenital infection and questions around re-infection and co-infection.
Purpose: Preliminary analyses to estimate the initial background sero-prevalence of dengue, chikungunya and Zika infections, incidence of new infections and outcomes in an ongoing prospective cohort study of asymptomatic and symptomatic pregnant women and their infants were done. The women were enrolled and followed in five public antenatal clinics, three delivery hospitals and two paediatric research clinics, by a multi-disciplinary team of clinicians and researchers, during June 2017 through March, 2018. The setting was the Greater Kingston Metropolis of Jamaica, a middle developing Caribbean island nation, with a population of 2.8

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million.

Methods: Data were prospectively collected on demographic, clinical, pregnancy and infant-related factors and complications. Serial fetal ultrasonograms were performed, blood and urine samples were collected on the pregnant women at eight-week intervals, placenta samples were also retrieved, with follow-up and sampling of infants. Serological testing for all three viruses was performed: anti-Zika IgM (IgM capture EIA, InBios International Inc and NovaTec Immundiagnostica GmbH) and IgG (EUROIMMUN AG), anti-Dengue IgM and IgG (PanBio) and anti-Chikungunya IgM and IgG (EUROIMMUN AG). The results of these laboratory tests were not used to direct patient care. Microcephaly was defined as head circumference less than -2 standard deviations below the mean for gestational age and sex (using WHO Growth Standards for term neonates and Intergrowth standards for preterm neonates). Approval was obtained from the Ministry of Health and University of the West Indies Ethics Committees. The women provided written informed consent for their participation along with their babies

Results: By the end of March 2018, 349 pregnant women had been enrolled and followed, 187 had second pregnancy visits, 56 had third visits and nine had afourth visit. There was one symptomatic (headache and joint pains) subject visit in the third trimester. There were eight pregnancy losses, including seven spontaneous miscarriages and one ectopic pregnancy. To date, there have been 201 deliveries, with 204 newborns (three sets of twins). There were 200 live births, four (2%) were preterm. There were four still births and five neonatal deaths [from placental abruption (two), preterm twins 28/40 with twin-twin transfusion (one), preterm (one) and perinatal asphyxia APGAR 1-1-3 (one)]. Therefore, the perinatal mortality rate was 44.1/1000 live births. Four (2%) live newborns were microcephalic. Of 258 first trimester antenatal sera tested, 177 (68.6%) were Zika IgG positive, 210 (81.4%) were chikungunya IgG positive and 256 (99.2%) were dengue IgG positive. Of the 429 maternal patient visit samples tested for IgM, sero-positivity for the arboviruses varied from 3 to 5.8%, with IgM sero-discordance between results obtained from two testing methods for Zika virus.

Conclusion: These initial 2017 to 2018 results provide important preliminary data on the background sero-prevalence of Zika, Chikungunya and Dengue in our cohort of pregnant women from the Greater Kingston Metropolitan region in Jamaica. Although some results are still pending (e.g. of molecular PCR testing and IgG sero-conversions), the few incident infections are consistent with our clinical and national arbovirus surveillance data, with breakthrough endemic arbovirus infections now being identified.

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Seroprevalence of asymptomatic infection by ZIKV in pregnant women in Rio de Janeiro

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Introduction: Several studies have demonstrated the association between cases of malformation and ZIKV infection during pregnancy. In the northeast region of Brazil, the first confirmed cases of ZIKV were recorded by the reverse transcription polymerase chain reaction (RT-PCR) method, in February 2015 (CAMPOS GS, et al, 2015).In August 2015, Pernambuco state reported the number of microcephaly cases increase, followed by a geographical and temporal association with the ZIKV outbreak, indicating the association between the microcephaly epidemic and ZIKV infection in women during pregnancy. In November 2015, the Brazilian Ministry of Health declared a state of national emergency. (FRANCE JVA et al, 2016; BRASIL.MS, 2015). Despite the increasing accumulation of evidences to the possible relationship between microcephaly and ZIKV, the effects of asymptomatic infections by ZIKV during pregnancy, especially in the first trimester, and congenital outcomes are not fully known, an essential characterization for the evaluation of the spectrum of congenital abnormalities. The risk of adverse events may be greater in symptomatic infections. However, asymptomatic infections are probably more frequent and consequently may significantly contribute to fetal health impacts. In addition, the investigation of asymptomatic cases in a population is essential to determine the transmission intensity and rate of infection of a disease. The objective of the study was to determine seroprevalence of asymptomatic infection by ZIKV in pregnant women from the Manguinhos, in Rio de Janeiro, from November 2015 to December 2017.

Methods: The inclusion criteria were women in any trimester of pregnancy assisted by the primary care teams from the community of Manguinhos followed by the Family Health Strategy program of the Prenatal and Child Care Services, from Nov 2015 to Dec 2017. Blood collections were performed after signing the consent form at the time of enrollment and every trimester until delivery. IgG (ELISA-panbio) for dengue was realized in all women. PRNT90 (seroneutralization by plaque reduction test) was the method of choice for the serological diagnosis of Zika: titers neutralizing antibody for ZIKV (> 240), was the cutoff point chosen for the reactivity.

Results and Discussion: 361 samples corresponding to 377 pregnant women have been analyzed. We observed that 30% (n= 111) of asymptomatic pregnant women had been infected by the ZIKV in the period. 286 women had already had dengue as evidenced by serology for dengue (IgG positive). PRNT90 has been used in the serological diagnosis for Zika, because it is still the golden standard. However, often it is unable to discriminate between primary and secondary infections between flaviviruses Zika and Dengue. Thus, the samples considered positive for dengue and positive by PRNT 90 for Zika will be submitted to the investigation of a possi-

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ble non-specific immunological response, since both flaviviruses simultaneously co-circulate, and could induce a cross response in the diagnosis even by PRNT, which is still a limited technique. For discrimination between zika and dengue of those sampes positive for both (dengue IgG and PRNT > 240), we will use Focus Reduction Neutralization Test (FRNT) for DENV-1, DENV-2, DENV-4, serotypes that circulated in Rio in the last 16 years. Choosing to be extremely specific we risk of having false negatives results, as not able to discriminate among the results of PRNT between 20 and 240, those truly negatives.

Preliminary experience in molecular biology for the detection of Zika virus in a cohort of asymptomatic and symptomatic pregnant women in Bucaramanga, Colombia

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The prospective multicenter observational cohort studies for evaluation of risks of congenital malformations and other adverse pregnancy outcomes after Zika Virus Infection, conducted within the European Commission (EC) Horizon 2020 (H2020)-funded ZIKAlliance Consortium in Latin American countries are ongoing since 2017. Molecular tests were carried out in a prospective cohort of asymptomatic and symptomatic pregnant women (PW), in Bucaramanga, Colombia, from June 2017. A total of 1213 samples (plasma and urine), collected from 200 PW recruited and monthly follow-up during the first 6 month of the study, were processed using Real Time RT-PCR for detection of Zika virus (ZIKV)-CDC Protocol. The preliminary results allowed to determine positivity in 1.9% (23 positive samples) with high Ct values ($\geq$36) that is, low viral load. During the reconfirmation, only one sample was positive. The analysis suggests that low Zika viral load may influence the non-reproducibility of the results. Additional tests, such as serological tests, are required to give a conclusive interpretation of these results.

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Cohort studies

Oral presentations

Do we still need the cohorts?

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The EC and other funders have invested into large multicentre cohorts in pregnant wome and children that are currently ongoing - but these cohorts operate under difficult general conditions as the incidence of Zika has declined in almost all countries in Latin America and the Caribbean. At the same time, first estimates of the risk of congenital abnormalities, including microcephaly, given confirmed Zika infection during pregnancy, were recently published.
Therefore, the provocative question is: Do we still need the ongoing cohorts - and if yes, for what?

In this key note, we will address the question what the ongoing cohorts can contribute to our understanding of Zika virus infection in pregnant women and children. Among other topics, the unexplained variability betwen the currently available estimates for the risk of microcephaly and other congenital malformations that are part of the Congenital Zika Syndrome (CZS) will be assessed. Other unresolved topics include the natural history of ZIKV infections in children who were evaluated normal at birth, and whether infections acquired shortly before or after birth also result in abnormalities, as well as the spectrum of abnormalities.
The currently ongoing cohort studies follow a number of objectives that can only be meaningfully analysed if data are shared and pooled at a later stage. The timelines implicated will be challenging as they are likely going to differ between the different cohort initiatives. The presentation will also give an overview about ongoing initiatives that rely on shared data being available at a later stage.

*Speaker

Zika and other arbovirosis in times of humanitarian crisis: The case of Venezuela

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Venezuela, once praised for its vector-control successes, has become a "heaven" for endemic and (re-)emerging vector-borne diseases to spread at an explosive rate. During the past four years, the country has plunged into a humanitarian and health crisis of increasing proportions. Economic and political mismanagement have precipitated a general collapse of Venezuela's health system with hyperinflation, people impoverishment and long term shortages of essential medicines and medical supplies.

In this context, the epidemic of Zika virus caused havoc among the population and on the national health system in 2016, which had not recovered from a previous arboviral epidemic, chikungunya. The impact of these epidemics was amplified by the lack of timely official information, lack of preparedness, and the worsening economic and health crisis. Both epidemics rapidly spread through densely inhabited regions where dengue transmission is high. The incidence of symptomatic cases during the peak of the epidemic was estimated as 2,057 x 100,000inh. Current estimates of serologically (IgG) Zika positivity in pregnant women reach roughly 80%. Venezuela was one of the first countries to report an increase in the number of cases of Guillain-Barré syndrome associated with Zika infection. Cases of microcephaly and other congenital disorders related to Zika infection in pregnancy have been reported, but the incidence is still to be determined by ongoing studies. In response to Venezuela's rapidly decaying situation, a massive population exodus is ongoing towards neighbouring countries and beyond. Emigrating infected individuals may unwillingly cause a spill-over of arboviral and other vector-borne diseases. With a government in denial of the current healthcare tragedy and neglect towards the reemergence of most arthropod-borne endemic diseases, a dangerous scenario is brewing for a possible epidemic of unprecedented proportions not only in Venezuela but in the region.

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First results from ZIKAlliance - Ultrasound monitoring of at risk pregnancies – how many Ultrasounds & when were they carried out

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Ultrasound (US) is an important diagnostic tool to assess the foetus and detect health problems, including severe anomalies that can be associated with the Congenital Zika Syndrome (CZS). Here we give an overview of the present state of ultrasounds carried out in the ZIKAlliance pregnant women (PW) cohort.
US is not a mandatory investigation in the PW cohort except for the US for gestational age to be carried out at the first visit of the PW cohort. The medically indicated additional US investigations including the anomaly scan are left at the discretion of the treating physician or are carried out according to national guidelines.

For the anomaly scan, we had created a standardized separate US CRF questionnaire, including a first part with general information and a second part with all the mandatory and optional features of the anomaly scan, stratified by before 13+6 weeks for first trimester and after 14 weeks for second and third semester.

In April 2018, 947 pregnant women from Brazil, Ecuador, Columbia, Venezuela and Bolivia had ultrasound done at least once, which amounts to a total of 2554 examinations. However, only 1244 of those examinations (605 PW) were performed as anomaly scans. 740 of all PW had ultrasound done more than once.

At this point, the mandatory fields were filled in 69,3% (depending on variable and country).

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There were abnormal US findings in 63 PW (10,4% of PW who had anomaly scans). The US of 10 PW (1,65%) showed signs of congenital abnormalities.
In the optional part, the degree of missing information was high with 70,9% for the first trimester variables and in 24,1% for the second and third trimester variables (ignoring the Doppler variables which start at week 24+0). To date, 3 fetuses (0,58% of all fetuses observed during second and third trimester) showed abnormal findings in the brain and 2 fetuses (0,39%) showed abnormal findings in the limbs. Due to the missing laboratory data, we do not know whether these women were infected.

Clinical outcome of congenital infections: lessons from a congenital CMV cohort

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Congenital cytomegalovirus infection (cCMV) is the most common congenital infection worldwide, with a birth prevalence of between 0.2 and 2.4 % in industrialized countries and between 0.6 and 6.1% in developing countries. cCMV was initially called "generalized cytomegalic inclusion disease" in the fifties. The first case series described infants with central nervous system involvement in the majority of cases. Many had microcephaly, psychomotor retardation or spasticity. The follow-up was usually short. In the sixties CMV infection could be reliably diagnosed trough viral culture of urine. However, the preferential testing of children with problems suggested that cCMV was a rare and severe often fatal congenital infection. The introduction of prospective screening revealed that the majority of infants with cCMV are asymptomatic at birth and that they generally have a favorable clinical outcome. Prospective cohorts have played a major role in understanding long term sequelae of cCMV. Recently, clinical outcome data of our large nation-wide retrospective cCMV cohort have been analysed. Pros and cons of retrospective and prospective cohort studies will be discussed and the impact of new clinical techniques on clinical definitions and strategies will be shown.

*Speaker

Lessons from imported cases

Oral presentations

Zika & pregnant women

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Objectives: To describe a case of a pregnant woman with Zika virus infection and severe fetal brain malformations.
Methods: Serial ultrasound measurements, fetal magnetic resonance imaging results, laboratory and amniocentesis results, and perinatal outcomes of the pregnant woman and her neonate are reported.

Results: Zika virus tested positive in amniotic fluid at 19 weeks while being negative at delivery. The newborn did not meet the case definition of congenital Zika virus syndrome because neither the Zika virus RNA nor immunoglobulin M antibodies were detected; however, prenatal brain lesions were confirmed after birth.
Conclusions: The presence of Zika virus in amniotic fluid plays a role in the diagnosis of Zika virus congenital syndrome

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Zika virus incidence and diagnostic tools accuracy in French international travelers visiting Latin America and the Caribbean during the summer 2016 -The ZIKAMERICA cohort study

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Introduction: The Zika virus (ZIKV) epidemic became a public health emergency of international concern in 2016 and a major preoccupation for travelers to Latin America and the Caribbean, especially in the context of the Olympic Games in Rio de Janeiro, Brazil, in August 2016. Three cohort studies focused on ZIKV incidence among international travelers to the Americas in 2016, i.e. two cohorts of Spanish and French travelers to the Olympics that failed to report any confirmed case after a screening by a first-line ELISA test (EuroImmun®) and one cohort of Belgian travelers to the Americas that presented preliminary results with incomplete follow-up in 2017. We aimed to estimate the incidence of ZIKV infection in French international travelers visiting Latin America and the Caribbean during the summer of 2016.

Methods: All the adult travelers attending a medical visit from June to September 2016 at the pre-travel clinic of Bordeaux (France) were invited to participate provided they planned to travel to Latin America and/or the Caribbean for a maximum stay of 3 months. Travelers with past history of ZIKV infection or journey to Latin America or the Caribbean from January 2015 to the date of visit were excluded. All the participants signed a written consent for the use of individual demographics, travel and health characteristics and for a 10mL blood collection 4 to 8 weeks after returning to France. A systematic diagnostic screening was performed with both the Euroimmun® antigen NS1-based ELISA IgM/IgG (EI) and the in-house ELISA tests of the CNR with IgM antibody capture (MAC) and indirect IgG with the use of complete virus lysate assay (INDG). A subsequent gold standard virus neutralization test (VNT) was performed for samples with positive or equivocal ELISA results or when Zika-like clinical symptoms were reported.

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Results: Of the 324 eligible travelers, 75 were included. Of them, 42 (56%) were women, with a median age of 34 years [IQR 29 – 55], 87% were tourists and 13% professionals. Median length of stay was 20 days [IQR 15 – 33]. South America accounted for 84% of travel destinations (31% Brazil, 21% French Guiana, 18% Peru), Central America plus Mexico for 8% and the Caribbean for 8%. Only 9% of the participants reported a visit to Rio during the Olympics. Half (38) reported at least one health event with 7 (9%) febrile illnesses and 12 (16%) non-febrile Zika-like presentations (7 rashes, 5 pruritus). Median sampling time after returning to France was 45 days [IQR 38 - 49]. Screening ELISA tests with EI IgM and MAC were all negative while EI IgG and INDG were positive for 4 and 1 travelers respectively. Three (4%) ZIKV cases were confirmed by a subsequent positive VNT, i.e. one woman with a history of febrile illness in Guadeloupe [EI IgG positive; MAC negative and INDG positive] and two women with non-febrile Zika-like clinical symptoms in Nicaragua, presenting with the respective [EI IgG positive; MAC and INDG negative] and [initial EI IgG negative; late EI IgG equivocal; MAC and INDG negative] serologic testing. The incidence rate was 4.4 ZIKV cases per 100 persons-months [IC95 1.1 – 12.1].

Conclusion: This cohort study is the first to report a complete follow-up with a significant ZIKV incidence rate among a population of international travelers exposed during an epidemic period. Both ZIKV cases with history of non-febrile Zika-like symptoms presenting with negative or discordant ELISA antibody response would not have been confirmed without the pugnacity of clinicians for obtaining a VNT. These findings emphasize the need already expressed in literature to promote new, reliable and rapid ZIKV serologic tests considering the lack of accuracy in the current ZIKV ELISA tests observed in both clinical routine and incidence studies for travelers to ZIKV endemic and epidemic areas.
The study is part of the Zikaplan project.

Serological response and clinical outcomes of children exposed to Zika virus during gestation: preliminary results of a prospective paediatric cohort study in a non-endemic country

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Background: Zika virus (ZIKV) infection has been associated to microcephaly and other neuro-developmental abnormalities. An ongoing Spanish database of ZIKV-exposed mother-child pair was created in May 2016. Our aim was to describe the first preliminary clinical/serological outcomes of ZIKV-exposed children followed-up in the main referral paediatric tropical medicine centres of a non-endemic country.
Methods: multicentric prospective observational cohort study of ZIKV-exposed mother-child pair (May 2016-January 2018). Children were recruited at birth and laboratory/clinical data from mothers were obtained from gestational database. ZIKV-infected mothers were defined as confirmed or probable following national guidelines. Epidemiological, clinical and laboratory data were registered on a RedCAP® database. Statistical analysis was carried out through Stataâv13. Ethical approval was obtained from participating centres.

Results: overall, 119 children (52.6% male) were included; median[IQR] gestational age at

*Speaker

birth was 39[38-40] weeks. ZIKV-infected pregnant-women (n=113) were from South-America 34.5%(39/113), Dominican Republic 37.2%(42/113), Central-America 24.8%(28/113), and other countries 3.6%(4/113). A 1.7%(2/119, CI95%:0.4-6.6%) of children presented at birth clinical/radiological signs of ZIKV Congenital Syndrome, up to 25% (CI95%:4.1-72.4%) for children born to ZIKV-confirmed mothers. Seven children showed audiological adverse outcomes (7/119, 8.1%; CI95%:3.9-16.3%) with mild/moderate hearing loss, congenital cytomegalovirus infection was ruled out at birth. All ZIKV-exposed children, except one, showed negative ZIKV results (IgM/RT-PCR) at birth. Among 12-m old children (n=39), serorreversion for IgG-ZIKV was achieved at a mean (SD) of 7.2 (3.7) months.

Conclusions: the largest series of prenatally ZIKV-exposed children in Europe estimated an overall ZIKV-congenital syndrome prevalence of 1.7% (CI95%:0.4-6.6%), up to 25% (CI95%: 4.1-72.4%) in the small group of children born to ZIKV-confirmed mothers. A considerable percentage of these children had adverse audiological outcomes (8.1%(CI95%:3.9-16.3%)), and a IgG-ZIKV serorreversion was achieved at a mean of 7.2 months after birth.

What did we learn from the European diagnostic labs?

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An emerging disease outbreak of the extend of the Zika virus epidemic challenges the ability of diagnostic virology centres who need to simultaneously implement and / or validate their diagnostic workflows for the new pathogen, assess what are essential knowledge gaps that are of relevance for test interpretation and clinical feedback, deal with the uncertainties raised by new disease associations, and handle the potential peak flow of diagnostic requests of the worried well. In Europe, the ECDC-funded laboratory network EVDlab net aims to provide state of the art diagnostics for rare and emerging (zoonotic) viruses, provide scientific advise on the pathogens and pathogenesis, and provide support for outbreak investigations at local, national, EU and global level. The Zika virus outbreak triggered a rapid assessment of critical knowledge gaps, a baseline survey of capacities and capability of laboratories of the EVDlab network, and external quality assurance assessment of the performance of the Zika virus diagnostics. The same approaches were offered to support the development of the Zika virus cohorts funded through the EU. The experiences from the EVDlab net and the collaboration with ZIKALLIANCE and ZIKAPLAN will be presented.

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Zika & adult travellers: Travel-associated risk for local transmission

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Screening of Zika virus infection in travellers:
We describe a group of travellers screened for Zika virus infection (ZIKV) in two centers of the International Health Program of the Catalan institute of Health (PROSICS, its acronym in Catalan), from January 2016 to March 2018. All of them were coming from Zika endemic areas and all of them were symptomatic.

We visited 223 travelers and Zika IgM and PCR were performed in serum, urine, semen /vaginal secretion and saliva when possible. We performed a total of 514 PCR with 42 positive results from 12 positive patients. Among the 223 travelers, only 24 were considered acute cases of ZIKV infection with positive IgM and/or PCR for ZIKV (10.7%).

Zika virus dynamics in body fluids and risk of sexual transmission in a non-endemic area:

We describe a prospective study at two centers of the Catalan International Health Program. The aim of the study was to assess the dynamics of ZIKV in several fluids of infected individuals through RNA detection and to determine the risk of sexual transmission to non-traveler sexual partners.

Methods: Symptomatic travelers diagnosed of ZIKV infection (positive IgG/IgM or positive ZIKV polymerase chain reaction (PCR) in blood/urine samples) were clinically followed up and ZIKV PCR was periodically performed in saliva, blood, urine and semen or vaginal secretion samples until they became negative, following the protocol study. Their sexual contacts were offered to participate.

Results: we included 11 travelers and 3 sexual contacts, 54.5% (6/11) were male. The median age was 38 (IQR 30-45). Travel reasons were visiting friends and relatives (6/11) and tourism (5/11). Median trip duration was 24 (IQR 11-34) days. Lab tests were performed 11 (IQR 3-31) days after symptoms onset. Nine out of 11 patients had a ZIKV IgM or PCR positive.One out of four women (25%) had a positive PCR in the vaginal swab 45 days after symptoms onset and 20% of men in semen up to 24 days. All patients with positive PCR showed positive IgG, while some patients with positive IgM never converted IgG.

*Speaker

Conclusion: ZIKV RNA detection was positive in genital fluids of 25% women and 20% men. Time for clearance was 37 and 24 respectively. No sexual transmission was found among sexual contacts. Diagnostic algorithms should be updated to include genital tract fluid specimens in the process. More research is needed to help public health agencies to inform more accurate recommendations.

Zika & microbiologic diagnostic challenges

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Zika virus (ZIKV) diagnosis is generally achieved by molecular detection of the viral genome and by IgM and IgG ZIKV-induced antibodies detection. Reverse transcriptase polymerase chain reaction (RT-PCR) provides a high sensitivity and specific diagnosis in ZIKV infection, but its use is limited by the short viremic period, and a negative RT-PCR result cannot rule out the diagnosis. There are some exceptions: a) when ZIKV viremia is extended, especially in pregnant women, as occurred in a pregnant woman with a congenitally infected fetus in which serum ZIKV-RNA was detected for 89 days; b) when ZIKV-RNA persists in genital secretions, as reported in a study about travelers performed by our group in 2016 with clearance time for ZIKV-RNA in vaginal and sperm samples of 37-69 and 23-107 days, respectively.
Serological diagnosis is challenging mainly due to cross-reactivity with other flaviviruses and may require the perform of plaque-reduction neutralization tests (PRNT). More specific serological assays such as ZIKV enzyme-linked immunosorbent assay (ELISA) has shown promising results compared to immunofluorescence (IIF) techniques. We compared IIF and ELISA from 212 serum samples of asymptomatic pregnant women. ELISA had better global results than IIF for the definitive ZIKV diagnosis (95.2% vs 62.2%). ELISA reduces costs and delay in ZIKV infection diagnosis because it's more objective and easier to implement in a laboratory routine.

Diagnosis in travelers: initially, ZIKV diagnostic algorithm relied on the presence of symptoms and time elapsed from the onset of them. Confirmed case definition was the detection of ZIKV-RNA in any biological sample and/or the detection of ZIKV-IgM plus a positive ZIKV-PRNT. Neutralization titers $> 1/32$ were considered indicative of the presence of ZIKV neutralizing antibodies. ZIKV diagnosis was obtained in 27/853 (3.2%) of our first screened individuals, 1/853 with positive ZIKV-IgM plus ZIKV-PRNT, 24/853 tested positive ZIKV RT-PCR, and 2/853 with both ZIKV-IgM and ZIKV-PCR positive.

Diagnosis in pregnant women: following regional public health guidelines, laboratory testing for ZIKV was universally recommended for all pregnant women with an epidemiological background linked to a travel history to ZIKV endemic areas during pregnancy. Overall, 1057 potentially exposed pregnant women were screened in two referral centers of Catalonia; ZIKV infection was confirmed in 14/1057 (1.3%; 95%CI: 0.7-2.2%) of them, leading to 128/1057 (12.1%; 95%CI: 10.2%-14.2%) when considering women with any laboratory ZIKV infection evidence (RT-PCR or serology with PRNT-ZIKV titer $> 1/32$). Among the 14 ZIKV-confirmed cases,

*Speaker

nine of them tested positive for RT-PCR in serum, three tested positive for RT-PCR in both urine and serum and two were diagnosed by serological methods.
Despite all the recent laboratory advances, ZIKV diagnosis still remains a challenge for clinical laboratories.

Zika: lessons from imported cases

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The pathogen Zika virus (ZIKV) generally results in moderate or clinically asymptomatic acute disorders. However, the 2014-2016 outbreaks in French Polynesia and America have focused attention on the impact of ZIKV on human health, as evidence accumulating that it causes severe neurological injuries and the potentially devastating complication of fetal infection due to transmission via the placenta. Travelers are integral to the global spread of ZIKV, serving as sentinel markers of disease activity. Surveillance of imported infections by returned travelers augments local surveillance efforts regarding ZIKV epidemiology and can assist with categorization by international authorities. Concurrently, travelers may facilitate further transmission. Hence, implementation of timely reporting of viremic travelers from areas with active ZIKV transmission is of concern among residents of areas with established presence of transmission-competent *Aedes* mosquitoes that calls for diligent vector control and vector and disease surveillance in order to prevent further autochthonous transmission. However, travel advisories are variable due to risk uncertainties. Besites, the risk of sexual transmission via semen of returning patients has been duly documented and is a real challenge for women of reproductive age and their partners. Serologic testing is a cornerstone for the diagnosis and screening of ZIKV infection in persons who travel in areas where ZIKV is transmitted. However, false negative results can occur using routine serological assays and can complicate the diagnosis of ZIKV infection and proper counseling of patients who are infected.

*Speaker

Social and ethical implications of Zika

Posters

The future status of genetic sequence-databases under the Nagoya Protocol: What can we learn from Culture Collections and Biobanks?

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In this year of 2018, the Conference of Member States (CoP) of the Convention on Biological Diversity / Nagoya Protocol discusses the status of genetic sequence data (GSD): do they fall by nature under the scope of the Nagoya Protocol or not? If GSD is to be considered as equivalent to tangible genetic resources (physical samples, as strains), the status of genetic sequence databases will be comparable to existing Culture Collections and Biobanks that already need to be compliant to the Protocol. Hence, how biobanks have coped with the Nagoya conditions and who bears the burden? What can we learn from it? We looked for systematic approaches for dealing with the Nagoya Protocol measures, and identified four models: the American, Japanese, Asian, and European. These models differ depending on how transfers of tangible genetic resources are performed for commercial and non-commercial use, if their redistribution is allowed, and who is responsible for the Access and Benefit Sharing negotiations with the country of origin of the resources. All investigated models end up being burdensome and compromising the timely sharing of genetic resources. The applicability of these models to databases is still questionable due to the intangible nature of GSD and the difficulties to track their access, transfer and use. Attention should be placed on the ability of biobanks to provide genetic resources promptly for public health emergencies by creating exemptions for pathogen genetic resources and/or facilitated systems of transfers. This would be possible only through the political engagement of national and international stakeholders daring to collaborate across their specific institutional mandates and domains.

*Speaker

Primary care pharmacist interventions in risk reduction for the zika virus epidemic: a study in Campo Grande, Mato Grosso do Sul Brazil

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Introduction: Pharmaceutical services for public health emergencies, such as the Zika Virus epidemic, are relevant for service effectiveness in the Brazilian Health System. Pharmacists can act strategically in risk reduction. However, official guidelines of the Ministry of Health do not consider pharmaceutical services when approaching health emergencies.
Objective: To identify and understand primary health care pharmacist interventions in risk reduction for the recent Zika Virus epidemic in Brazil.

Methods: The study took place in Campo Grande, Mato Grosso do Sul, in November, 2017. A semi-structured questionnaire was developed, including general issues related to knowledge of Zika, risk communication, and the pharmacist's role in patient care for Zika Virus disease. The instrument was pre-tested. PHC pharmacists were subsequently interviewed. Aspects related to knowledge, risk reduction measures and role were categorized and analyzed. The project received approval form the IRB at the Sergio Arouca National School of Public Health.

Results: Forty-two of the 48 PHC pharmacists in Campo Grande were interviewed. Risk reduction measures were cited by most interviewees. Among these strategies, 92% were collective measures, such as making information available for the population (30%) and for the health workers (8%), and vector control strategies (43%). Use of mosquito nets was the most cited individual risk-reduction strategy. Only one pharmacist cited risk for pregnant women, and suggested birth control as a strategy. Another pharmacist pointed to ZIKV 'treatment'. No interviewee mentioned measures related to preparedness of pharmaceutical services.
Conclusions: PHC pharmacists do not place themselves at the front line of risk reduction for the Zika Virus epidemic. In face of potential hazards and consequences of this disease, pharmacist actions, as health professionals, is deemed critical. This study highlights pharmacist's misconceptions and lack of focused knowledge, pointing to the need for training and capacity

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building in order to increase quality of care and positive management of future epidemics.

The microcephaly children visibility and the Brazilian government responses: clues to the care production process through the epidemiological bulletins mapping

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This work aims to discuss some of Brazilian government responses to the zika virus epidemic, as it becomes a risk to the national public health future through microcephaly babies' birth. We concern with children affected by zika virus' care insofar the first glances of the epidemic with the intention of mitigating this effects were not the production of caretaking but the improvement of both epidemiological surveillance and actions on combating the *Aedes aegypti* mosquito. We inquire if those children became as visible as the disease in order to demand prompt government solutions to their problems. Also, if the epidemiological surveillance headcounts activities were able to promote the State's production of operative actions. Our source of material is the *corpus* of 24 Microcephaly Epidemiological Bulletins (MEB) produced by the Brazilian Health Ministry as a tool of monitoring microcephaly cases, considered here as "documents of emergency". The Epidemiological Bulletin (EB) is a Sanitary Vigilance Department's (SVD) electronic format for results publication on specific diseases' monitoring and investigation. The MEBs published during the year of 2017 stand out from others EB as there is an entire section destined to recapitulate all previous documents "elaborated and published" by Health Ministry on zika and microcephaly. The sense of temporality to the epidemic set out by those documents organize this work, in order to map out some of the official measures and to seek for concepts and strategies that guided both the construction of the government responses and the sense of uncertainty and emergency that pervaded this massive document production. The first 2015 formal guidelines set up that all confirmed microcephaly babies ought to get specific pediatric primary care and professional early stimulation and, as babies might present some important specific changes, follow-up by different specialists would be necessary. On the other hand, of the 542 confirmed cases published in the 2017 MEB, only 37,6% children were in specific pediatric primary care, 18,5% received professional early stimulation and 33,9% were in specialized attention attendance. Besides, merely 45,6% babies were at least in one kind of care; 12,5% were receiving both specific pediatric primary care and specialized attention attendance; and 13,8% were receiving the three types of attention associated. Soon after the 2016 WHO PHEIC declaration, the Health and the Social Development Brazilian Ministries published three Interministerial complementary documents aiming to strengthen the health care and the social protection of children with microcephaly. One attachment action between the two sectors was the accomplishment of a medical report in order to plan the individual care and to instruct the granting process of the

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Continuous Provision Benefit. This Benefit is the guarantee of a monthly minimum income for the disabled person who has to prove the lack of means to provide his own maintenance or to have it provided by his family. Only children victims of microcephaly due to neurological sequelae resulting from diseases transmitted by *Aedes aegypti* are entitled to receive for a maximum period of three years, as persons with disability. In addition, though on 2017 the epidemiological surveillance began to perform an integrated monitoring of the changes in children's growth and development due to zika virus infection and other etiologies, the Interministerial intervention framed the health and social actions exclusively on microcephaly. This means that the State increasingly identified and notified disabled children without the same production of caretaking improvement. In this way, the official interference process made the microcephaly offspring more visible than the majority of children affected by zika virus inasmuch it framed to microcephaly concept the strategies that guided the construction of the government responses. Thereby, the processes created conditions for the constitution of particular interests groups within the bigger assembly of both those affected by zika and those of disabled people, as well as increased the means of building the iniquity in the nation through the microcephalus existences.

"What do you know about Zika?" Investigating women at primary health care in a small municipality in Brazil

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In Brazil, poverty-stricken population groups were the most affected by Zika. Women and children are fragile links that need focused attention, especially in relation to health care. The aim was to investigate vulnerable at-risk women in relation to their awareness of the ZIKV infection and on knowledge about the disease.
Based risk communication literature and consequences of ZIKV infection a data collection instrument with open-ended questions was developed. Women, from a small municipality in the West-Central Region of Brazil, many from a rural setting, were interviewed at PHC centers, in April 2018. All interviews were recorded and transcribed and a preliminary analysis ensued.

Overall, 40 women were interviewed. Average age was 42.3 (21-74 yrs) and 39 women had at least one child. The average number of people living in the same home was 3.8 (1-18) and 24 homes (60%) harbored 1-4 children. Fourteen women (54%) were beneficiaries of income supplementation programs. Two interviewees mentioned they had never heard of Zika and 8 (20%) had no actual knowledge to convey. Other groups had some knowledge about ZIKV; 15 (37.5%) associated ZIKV with mosquito bites and another 15 with pregnancy or birth defects. Ten women (25%) mentioned dengue or chikungunya but only 7 (17.5%) were aware of symptoms. Only eleven women (27.5%) declared public health workers as information sources.
Positive aspects of awareness and knowledge were the tentative relationship some women made between pregnancy risk and exposure to mosquitoes, and with dengue or chikungunya. However, given ample media coverage and severity of the epidemic, it is noteworthy to point out that all aspects were mentioned by less than half of the women. Health workers were not represented as relevant sources of information. Future in-depth content analysis of interviews may reveal important issues for risk communication strategies for this population.

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Memories of the Zika virus epidemic in Brazil: risk perception and response strategies from an anthropological perspective

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This poster aims to present post-epidemic narratives about the spread of Zika virus occurred in Brazil between 2015 and 2016. The circulation of medical information and rumours during the epidemic led to an accumulation of scientific and lay knowledge by different actors (physicians from northern countries and southern countries, health community centre actors, pregnant women and women of childbearing age etc.). As such, it had influenced reproductive life and changed practices in some instances. I will present my first results based on an ethnographic field driven in September and October 2017 with women of childbearing age in São Paulo and Recife. The changes in reproductive life can be understood through the dual aspect of *risk perception* (and *fear* of severe outcome in new-born) and *response strategies*. Both aspects depend on women's location, previous experience of vector-borne disease, social class, and access to health care and prenatal care (public or private). Consequently, I also intend to discuss how a global health emergency is perceived at a local level in a context of great medical uncertainty and after the proclaimed end of Zika virus epidemic.

*Speaker

The role of civil society response to Zika in Brazil: addressing neglect through community-centred approaches

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The ongoing Zika epidemic has revealed the fragilities of Brazil's public health system. It has shown the extent to which the lives of Brazilian women – and particularly those from poorer backgrounds – are marked by long-standing vulnerabilities. Many women in Brazil are still unable to access the best available standards of healthcare and the most up to date information about risks. Zika, and the associated growth in the incidence of microcephaly in newborn children, has raised issues pertaining to reproductive health rights in a country where abortion is illegal. The presence of microcephaly and other complications also means that the Zika epidemic is a health crisis in the medium- and long-term. This research collaboration sets out to address an important gap in existing Zika-related research: an engagement with marginalized women's perspectives. It does so by promoting a sustained partnership with twelve social movements representing traditionally-neglected sectors of the population of the state of Minas Gerais, the second most populous in Brazil. The objective of this engagement is twofold. First, the project sets out to achieve a detailed perspective of the challenges posed by Zika and microcephaly to women of vulnerable backgrounds. By way of extensive fieldwork, the research team aims to provide a rich and nuanced bottom-up perspective of the challenges raised by Zika and microcephaly. Second, by establishing a bridge between civil society, policymakers and health professionals, the project aims to provide a forum in which public policy can be discussed between all relevant stakeholders. Benefiting from privileged access to both civil society actors and policymakers in the state of Minas Gerais, this collaboration ultimately seeks clear and realistic recommendations that will improve the quality and impact of public health policies in Brazil.

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Scientific collaborations in Zika: identifying the main research groups through Social Scientific Network analysis

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Brazil was the center of world's attention during Zika epidemic due to the expressive number of microcephaly occurrences in new-borns. Science was not able to explain how a virus with no medical importance till then, became the cause of a public health *emergency of international* concern, a global health threat. Scientific explanations and control measures were of upmost importance. Since then, collaboration among health institutions from all continents were established and a very expressive body of knowledge was developed.
Based on this assumption, this group mapped the social scientific network on Zika, showing how researchers of different countries, institutions and expertise worked together to develop studies and protocols that would lead them to major scientific breakthroughs, which groups were the most productive and who were the more influent researchers in the study of Zika.
To this purpose, methods in Social Network Analysis were applied to study the co-authorship networks developed between the years of 2015 and 2016. Data obtained from this study show that the influence of a researcher is based on three factors: the number of scientific articles he publishes; the diversification of partnerships he establishes, and the links established with the pioneers in the field. We could also observe that networks have strong geographic reference, and despite the large amount of Brazilian researchers involved in cohort studies and in international networks, they are rarely first authors in the scientific articles.

*Speaker

Social and ethical implications of Zika

Oral presentations

Health promotion in the context of the Zika epidemic: actors and scenarios in decision-making process

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Since 2015 the Brazilian population lives with the social repercussions of Zika Virus (ZIKV) epidemic, which raises debates about difficulties of diagnosis, access to care for children with congenital Zika virus syndrome (SCZ), search for benefits, social and gender inequalities, reproductive rights, among others.
Objectives: This study aims to apprehend the dimensions of health promotion in the light of the epidemic of Zika in contexts of vulnerability, recognizing social markers of gender, race, social class of residence and religion.

Methodology:Exploratory analysis of reported cases of microcephaly from 2015 to 2017 related to the presence Zika vectors as a risk proxy in Brazillian municipalities. Case study analysis of social and territorial vulnerability of the States of Bahia (BA), Paraiba (PB) and Rio Grande do Norte (RN) were the epidemic was particularly severe. Spatial analysis of selected indicators from official databasis and fieldwork with aplication of in-depth interviews and focal groups in the cities Feira de Santana (BA); João Pessoa (PB) and Natal (RN). Health coverage and access to CSZ diagnosis and treatment, social vulnerabilities indicators and responses to the epidemic were mapped using Health State Secretaries' protocols and official databases of the Brazillian Statistics Office (IBGE) and Ministry of Health (DATASUS). Fieldwork in Feira de Santana (BA) comprehended 23 in-depth interviews and 3 focus groups with parents of children with CZS, men and women in reproductive age and health professionals involved directly with Zika patients. The research instruments were previously tested and validated by the Brazilian Ethical Comity - CEP/ENSP N. 67311617.8.0000.5240.
Results: Simultaneous circulation of Zika, dengue and chikungunya virus, especially in the Northeast region of Brazil made difficult the analysis of reported Zika cases. However there was an association between states with high infestation of *Aedes aegypti*and incidence of microcephaly. The distribution of microcephaly cases from 2015 to 2017 shows a undeniable concentration in the Brazillian Northeast. Rio Grande do Norte presented the highest incidence of microcephaly in 2015 and Paraíba in 2016. Difficulties of entomological control of the *A. aegypti*and social vulnerability of the Northeast urban population creates a difficult scenario for poor families affected by CZS obtaining diagnosis and treatment. Coverage of prenatal exams is satisfactory in most municipalities of the study area, but availability of ultrasound equipment is

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uneven, specially Bahia's western region. The first fieldwork in Feira de Santana (BA) revealed situations of embarrassment and difficulties faced by mothers of children with CZS in their caring trajectories. Respondents reported lack of sensitivity in dealing with microcephaly prognosis. Men and women in reproductive age were confused about Zika and dengue consequences; in the poor quarters without health agents there are no prevention actions. There are complaints about low participation of men on caring for children with CZS, including household abandon. Nevertheless, health professionals try to engage fathers in the care of their children. Further investigations will be held in Natal (RN) and João Pessoa (PB), as well as the spatial analysis of other vulnerability indicators. This study was supported by the European Union's Horizon 2020 Research and Innovation Programme under ZIKAlliance Grant Agreement no. 734548.

Zika and the state's policy trail: a document review on the Ministry of Health's responses

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This paper aims to look at the production of official documents as one of the main responses given by Brazil's State governement, namely the Health Ministry. This federal institution has been historically responsible for creation of the country's public health policies and scientific productions. We follow Das suggestions (2007) to see the State's authority through thinking in terms of literality inscribing a sense of temporality, as well as policies and actions. This intensive production of State's literacy also relates to an ongoing process of scientific meanings and concepts stabilization, construing official narratives. They are documents that construe temporalities, produce marks and silences, leave trails and inscribe some sense of official history. It was conducted a document analysis based on Brazilian ministry of health legislation in the period from January 2015 to December 2017. The data is available at the Ministry of health's website.

We approach the corpus of documents having in mind the possible relations between the political and its intersect with scientific responses paying special attention to uncertainties, the production of visibilities and invisibilities that surrounded – and still does, this disease. One of our main attempts here is to map out not only the uncertainties, the known and the unknown inscribed in the document's temporality but also what and which zikas are being "enacted" (Mol. 2002).

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The cognitive and emotional representations of Zika and other mosquito-borne diseases in French Guiana

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Human behaviors are increasingly recognized to play a key role in the spread of infectious diseases. Although a set of emotional and cognitive representations has been consistently found to affect the adoption of health protective behaviors aiming to control and prevent a variety of infections, little is currently known about these determinants in epidemic settings. In 2016 we took advantage of the outbreak of ZIKAV infection that occurred in French Guiana to characterize the representations associated with several mosquito-borne diseases. A cross-sectional phone survey was conducted among the general population in June 2016 with a total of 1,129 individuals interviewed to assess mental representations and behaviors regarding ZIKAV infection. Overall, the population seemed aware of the Zika virus threat, and perceived the infection as a more serious health threat than other common mosquitoborne diseases. Furthermore, both the perceptions and behaviors related to zika and its prevention were found to vary considerably among different social groups, geographic areas and gender; less educated female participants were found to perceive the disease as more worrying and were less likely to adopt protective behaviors.

*Speaker

Production and circulation of knowledge about Zika: from scientists to social media users

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Scientific production, risk communication, repercussions on the health system and social assistance, social and environmental determinants of health, living conditions, sexual and reproductive rights are important issues highlighted by Zika outbreak when it comes to what has circulated online. To social sciences, investigating this phenomenon has demanded new strategies of collecting/crawling, organizing/visualising, understanding/analysing big amounts of raw data. The challenge has been transforming social researchers used to deal with small amounts and single-source data on interpreters of big "multi-sourced" data, without giving up the qualitative in-depth analysis of what can be extracted from them to address multifaceted research questions around a phenomenon, like an epidemic.
In this study, we aimed, among other goals, to investigate how knowledge about the infection, practices of care, and social imaginaries of disease are co-evolving during the first three years of the Zika epidemic in Brazil (2015-2017) from the perspective of science and the general public. The research team mapped, mined and analysed different kinds and sources of data: scientific publications, press releases, the content of social media websites, different engaged civil societies, academic institutions, research teams, and other stakeholders. To each of them, specific methodologies and tools in a digital method approach were combined to extract and analyse data, considering the mixed methods perspective of this investigation.

Data grabbed pinpoints clusters and groups of people who can represent the public who engages to this theme online, exploring the circulation of knowledge amongst the main actors and investigate their centrality on engagement networks. Outcomes have, so far, pointed to four different "zika epidemic" for further analysis. 1- the epidemic on press; 2- the epidemic expressed by a network of scientific publication and circulation of knowledge on Zika 3- an epidemic discussed and depicted by the content and visual cross-platform engagement on social media by the general public 4- the information and expertise present on the discourses of people from different regions and social realities in Brazil.
Connecting these results in a map of meanings of the epidemic produced from the content about Zika shared worldwide, allows a better understanding of the dynamics of knowledge production, circulation and assimilation with regard to epidemics like Zika amongst the general public can

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help in the development of improved communication strategies for diffusion of biomedical and public health knowledge.

Narratives on Zika epidemic in Brazil: Challenges to science, politics & society

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The Zika epidemic in Brazil produced different narratives on perceptions, meanings and reactions. Based on the concept of Mol (2002) this work aims to identify the zika's enactments during and after the outbreak in Brazil from 2015 to 2018. We analysed the narratives emerged on documents, science uncertainties, health policies responses and social movements statements. The discourses and practices analysed in narrative productions in Brazil were marked by visible and invisible processes, reductions on prevention policies, and ethical issues on scientific procedures related to families and children with zika congenic syndrome. The lessons learned by Zika outbreak responses and its consequences in Brazil point out the demand to improve listening to social movements needs and participation in science and policy-making process during public health emergencies, an interdisciplinary and intersectoral approach to respond to social inequities in health access and life conditions responsible for many neglected diseases and vectors dissemination around the country. In a context of decreasing investment in science and technology, and reducing social protection policies funding in Brasil, the threat of new Zika outbreaks and other arboviruses demand a strong reaction of national and international scientific communities to defend the right to health for all again.

*Speaker

Zika Outbreak in Brazil: Challenges for Science, Public Health, and Society”

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Global public health was surprised by an unknown disease and its terrible consequences. In September of 2015 began an increase never seen in cases of congenital microcephaly that was later associated with an epidemic of Zika virus in Brazil. This historical fact produced a paradigmatic impact in the field of public health and biomedical sciences only compared to the AIDS epidemic in the 1980s and Ebola in Africa in the year 2010. The unforeseen consequences of the Zika epidemic in Brazil required the national health system (SUS), Brazilian scientific institutions and the international community a fast, efficient and reliable response. This presentation aims to discuss the challenges posed to science, public health and society in the face of the Zika epidemic and its historical, social and political determinants.

*Speaker

Repercussions of Zika Virus Epidemic on the National Health System in Brazil and households

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Introduction and aims: The goal of our study is multifold: to evaluate the response of the Unified Health System (UHS) and health services in facing the epidemic, considering the dimensions of health policy, health care, and the control, protection and prevention strategies; the economic repercussions of the epidemic from the standpoint of families who have children with congenital zika syndrome, considering private economic costs, measuring the possibility of catastrophic expenditures and the risk of impoverishment of households. Problems related to organization of health delivery, access and use of the public health system by affected individuals, as well as indirect costs for families, and the estimation of indirect costs.
Methods: Design comprises the analysis of multiple cases, involving the federal, state and municipal health spheres, considering the period between 2015 (beginning of the identification of the zika epidemic) to 2017. Two data sources are currently being used: documents and interviews with managers; interviews with families of children with congenital zika syndrome (structured instruments with closed and open-ended questions and based on a qualitative approach, using an instrument with open-ended questions).

Results: During the health emergency, the main responses of the UHS were: mobilization and mosquito control; people care and technological development, education and research. The actions and strategies were defined based on emerging information and demands, being reviewed periodically. It is important to highlight the relevance of reference care services for the care of affected children and pregnant women, research institutions and diagnosis network, the intra and intersectoral articulation as well as between different government levels. In surveillance and healthcare, the infrastructure and organization of the available services were utilized, without major investments, expansion or reorganization of the network. Regarding households and families, our findings indicate catastrophic health expenditures and great productiveness losses for parents of affected children.
Conclusions: After the sanitary emergency there were many demands to be met. It is necessary to reorganize and invest in the healthcare and laboratory network of the UHS to care for those affected and others who may be affected by the disease and its effects in the short, medium and long terms. Intersectoral action and reduction of inequalities are key components. It is essential to provide a safety net for families with affected children through policies and targeted initiatives. Lessons learned should support future responses to new arboviruses.

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The sharing of pathogen genetic resources under the Nagoya Protocol: What are the ethical implications for international research on the ZIKAV?

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ZIKAlliance has as its overarching goal to implement sustainable collaborative clinical and scientific networks to address past, current and future ZIKAV epidemic threats. For that, the rapid sharing of viral samples and its genomic sequences is an essential element to facilitate the development and deployment of disease detection methods and state-of-the-art diagnostics for case finding and contact tracing. However, since October 2014 the international treaty known as the Nagoya Protocol (NP) has the potential to limit public access to, and the free exchange of, pathogen materials. In international collaboration, scientists can be caught in an ethical dilemma of the urge of providing the tools for essential health care on the one hand, and compliance to benefit-sharing-regulations on the other. The combination of partial implementation of the NP, lack of clarity on rules and processes, and differences in interpretation at national level are foreseeable major hurdles, especially in outbreak situations, as the one of ZIKAV, due to probable delays on the sharing of and access to pathogens and their sequences. ZIKAlliance, as a multinational and multi-disciplinary research consortium, is engaged in intercontinental epidemiological studies involving countries in Latin America and the Caribbean, Europe and partners in Africa, Asia, and Polynesia. Many of these countries are party to the Nagoya Protocol. The consequence of which is that the collaborative research partners have to exercise due diligence by acquiring specific documentation and have to negotiate bilateral contracts with country's governments for the use of each isolate of the many samples obtained from the different countries. Additional to the NP-status of tangible materials, the Conference of Parties of the Convention on Biological Diversity and the Nagoya Protocol initiated in November 2017 the discussion on the status of genomic sequence data from viral and microbial isolates: do they fall by nature under the scope of the Nagoya Protocol or not? If yes, what will be the impact for clinical and public health research? Which solutions for free, fair and fast sharing of essential pathogen information can be proposed to promote flexibility and global harmonization of protocols in order to anticipate, follow and respond to global outbreaks efficiently? Such questions, building on results from previous research on the topic, will be brought to the attention of the ZIKAlliance conference for further discussion; and participants will be invited to share their experiences with the sharing of materials and data.

*Speaker

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Other important questions

Posters

Sexual transmission of Zika virus – estimating the duration of infectiousness

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Introduction: Zika virus (ZIKV), primarily spread through infected mosquitoes, can also be transmitted between persons through sexual intercourse. Sexual transmission of ZIKV is of special interest because of the risk of congenital abnormalities, such as microcephaly. Health agencies have produced guidelines on the prevention of sexual transmission of ZIKV, but the risk of transmission is incompletely understood. In particular, the duration of infectiousness has not been directly estimated; instead the duration of RNA detection in bodily fluids has been used as proxy for infectiousness. Here, we summarise evidence about the risk of sexual transmission of ZIKV and the different measures of duration of infectiousness.
Methods: We did a systematic review of evidence on the sexual transmissibility of ZIKV up to April 15th 2018. We included observational studies assessing the persistence of viral RNA and culturable virus, as well as studies describing sexual transmission in couples. We aggregated the duration of persistence of viral RNA and culturable virus in semen and the female genital tract, using interval censored survival analyses. We compared the estimates reported from prospective cohort studies with the estimates we calculated using the data from case reports and case series.

Results: We identified 1217 unique publications and included 48. Sexual transmission was reported in 36 human couples: 34 were male to female, one female to male, and one male to male transmission episodes. The median serial interval, available for 15 couples, was 12 days (interquartile range, IQR: 10-14.5 days). Estimates of ZIKV RNA persistence in semen were: median 34 days (95% confidence intervals, CI: 28-41 days) and 35 days in two cohort studies; and 40 days (95% CI: 30-49 days) based on data from case reports and case series. The maximum duration of ZIKS RNA detection was 370 days. Culturable virus in semen could be detected for a median duration of 12 days (95% CI: 1-21 days), with a maximum of 69 days. In the female genital tract viral RNA was detected for a median of 14 days (95% CI: 7-20 days), with a maximum of 37 days. The quality of the evidence is limited because of uncontrolled residual bias and indirectness.
Conclusion: Sexual transmission of ZIKV is more likely from men to women than from women to men. Studies about the duration of detection of ZIKV in bodily fluids and the serial interval suggest that the period of ZIKV infectiousness through sexual transmission might be shorter than was anticipated from the earliest studies in 2016. Early estimates of duration of persistence have likely overestimated the true duration of persistence.

*Speaker

Other important questions

Oral presentations

Sexual transmission of Zika virus: the current state of affairs

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Zika virus (ZIKV) can be transmitted via sexual intercourse. Among arboviruses, this seems a unique feature. Surveillance reports, clinical case and cohort studies and experimental data from animal studies have contributed to rapid knowledge gains, but many uncertainties remain. A synopsis of the knowledge of sexual transmission of ZIKV anno 2018 and its determinants will be presented and its impact on the burden of ZIKV disease will be discussed. Knowledge gaps that warrant further research will be identified.

*Speaker

Potential effect of Zika virus infection on human male fertility

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Background: Zika virus (ZIKV) sexual transmission and prolonged viral shedding in semen have been previously reported, suggesting strong viral affinity for genital tissues. A transient impact of ZIKV on male fertility was shown in animal and human studies.
Case presentation: Adult male patients with confirmed ZIKV infection diagnosed during the epidemic season of 2016 were invited one years after acute infection to respond to a questionnaire of genital symptoms and to provide a semen sample for molecular ZIKV testing and spermiogram analysis, as well as a serum sample for hormonal testing. We report abnormal spermiogram results from patients one year after confirmed ZIKV infection.
Conclusions: Our findings suggest a possible long-term detrimental effect of ZIKV infection on human male fertility, to be further explored in well-characterized samples from cohort studies conducted in ZIKV-endemic areas.

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Zika virus infection perturbs osteoblast function

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Zika virus (ZIKV) infection is typically characterized by a mild self-limiting disease presenting with fever, rash, myalgia and arthralgia. Virus-induced arthralgia due to perturbed osteoblast function has been described for other arboviruses. In case of ZIKV infection, the role of osteoblasts in ZIKV pathogenesis and bone-related pathology remains unknown. Here, we study the effect of ZIKV infection on osteoblast differentiation, and function by quantifying activity and gene expression of key biomarkers, using human bone marrow derived mesenchymal stem cells (MSCs, osteoblast precursors). We found that MSCs were highly susceptible to ZIKV infection. While infection did not cause a cytopathic effect, a delay in osteoblast differentiation and function was observed as compared to uninfected controls. In conclusion, we have developed and characterized a new in vitro model to study the pathogenesis of ZIKV induced arthralgia. This will help to identify possible new targets for developing therapeutic and preventive measures.

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Improving serological diagnosis of Zika virus to understand NeuroZika in Brazil

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Background: During 2015–16 Brazil experienced the largest epidemic of Zika virus (ZIKV) ever reported. This arthropod-borne virus (arbovirus) has been linked to Guillain-Barré syndrome (GBS) in adults but other neurological associations are uncertain. The increase in congenital defects and other neurological diseases associated with ZIKV infection in Latin America has highlighted the importance of highly accurate laboratory tests for managing cases. The WHO recommends that diagnosis of ZIKV seven days after symptoms onset should be performed with serologic assays. Assays that detect ZIKV antibodies are available but the performance of the assays is significantly affected by prior exposure to other flavivirus because of extensive antigenic cross reactivity. We investigated the use of ZIKV diagnostics with adults with acute neurological disorders for ZIKV, chikungunya and dengue, another arbovirus circulating in Brazil and we evaluated the performance of a range of ZIKV assays with various populations in Brazil including neurological cases.
Methods: We investigated adults who had developed a new neurological complication following suspected arboviral infection between 1st November 2015 and 1st June 2016. We also performed the evaluation of 4 ZIKV IgM assays: a commercial Zika-NS1 IgM ELISA, an in house capture assay (MAC-ELISA), a commercial capture assay (Novagnost) and a rapid IGM test. Samples were referred for routine diagnostics to the reference Flavivirus laboratory at the Institute Oswaldo Cruz, Rio de Janeiro. Sensitivity was measured using a panel of sequential serum samples collected in 2015-2016 from 67 ZIKV PCR positive cases (n=159). Specificity was assessed using sera from subjects with confirmed (PCR and IgM positive) exposure to Dengue (n=89), yellow fever (n=19); collected before 2013 to exclude potential ZIKV exposure as significant ZIKV transmission started in Rio in January 2015.

Results: Of the 35 patients studied, we found evidence of recent arboviral infection in 22 of them. Twelve had positive PCR or IgM for Zika. Regarding the evaluation of diagnostic assays, our findings show that of the ZIKV IgM methods evaluated, the IgM Novagnost has a better overall accuracy (sensitivity and specificity) compared to the others. We also demonstrated a large proportion of false positive results in the Dengue assays following ZIKV infection.

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Conclusions: ZIKV is associated with a wide range of neurological manifestations, including central nervous system disease to understand the burden of Zika we must look beyond GBS. Our findings also highlight the importance of using representative samples from the local population for validation to guide the selection of optimum tests and reinforces the need for more accurate diagnostics, especially for neurologic ZIKV patients presenting after the acute phase of infection.

The gender gap in the ZIKV infection: A social & behavioral perspective

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Differences in Zika prevalence by sex are an interesting but uneven finding of the epidemic that have yet to be fully explained, in common with many other infectious diseases. Whilst some work has been done on immunological differences by sex, little research has focused on possible gender-based behavioural factors that may affect exposure and consequent prevalence. As with both sociocultural and behavioural contributions to disease dynamics, particularly those focusing on sex differences, there remains much work to do to better understand possible mechanisms involved. Difficulties also include how to integrate social and biological data, and whether approaches such as cultural epidemiology can be of use in this area. This presentation will discuss the current data available on differences in Zika prevalence between men and women from a social and behavioural perspective, exploring possible mechanisms for this difference in contrast with immunological factors and sampling bias. The gap between sex-based differences as biological and possible gender-based differences as biosocially constructed within epidemics will also be elaborated on. Additionally, the current state of understanding on the gender gap in Zika, in comparison to other vector-borne viruses such as dengue and chikungunya will be presented, including the possible role of sexual transmission in prevalence differences by sex. This will enable some discussion on potential approaches to better understand possible linkages between behavioural factors, specifically by gender, and epidemiological patterns, by sex.

*Speaker

Zika virus infects the human testis and germline

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Zika virus (ZIKV) is a teratogenic mosquito-borne flavivirus, which can be sexually transmitted from man to woman. High viral loads and prolonged viral shedding in semen suggests that ZIKV replicates within the human male genital tract, but its target organs are unknown. Using *ex vivo* infection of organotypic cultures, we demonstrate here that ZIKV efficiently replicates in the human testis and infects a broad range of cell types, including germ cells, which we also identified as infected in the semen from ZIKV-infected donors. ZIKV had no major deleterious effect on the morphology and hormonal production of the human testis explants. Infection induced a broad antiviral response but no interferons up-regulation, and minimal pro-inflammatory response in testis explants, with no cytopathic effect. Finally, we study ZIKV infection in mouse testis, and compare it to human infection. This study provides key insights into how ZIKV may persist in semen and alter semen parameters, as well as a valuable tool for testing antiviral agents.

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Latest Zika virus outbreak: improve preparedness of disease control from the perspective of the "European Virus Archive goes Global (EVAg)" EU funded consortium

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Zika virus (ZIKV) has emerged in the Americas in February 2014 on Easter Island, Chile, and has since been causing an unprecedented outbreak in several countries in the Americas. In May 2015, PAHO issued an alert for the first confirmed case of Zika virus infection in Brasil, and on the 1st of February 2016, WHO declared a Public Health Emergency of International Concern (PHEIC). In this context, the "European Virus Archive goes Global", EVAg, a non for profit EU consortium grouping virology laboratories worldwide, distributed more than 300 ZIKV related products. The main mission of EVAg is the development and maintenance of a large biological resource of authenticated viruses and related products in order to facilitate their access to both academics and industries. Together with this mission, one of the EVAg goals is also to support Public Health response during viral outbreaks. The Zika outbreak EVAg coordinated response was to propose a set of products available via its online catalogue. WHO in its interim guidance for ZIKA laboratory testing (March 23, 2016) identifies EVAg as the provider for PCR quality control material (Zika standards for use in molecular detection).A unique web based entrance for the request of viruses and derived products developed by several EVAg members allows a retrospective analysis of the demand. During the ZIKV outbreak, more than 300 products were distributed worldwide, the majority in the first trimester of 2016, with a peak in February, immediately following the WHO PHEIC declaration. We will present a thorough analysis of the EVAg products end-users, their geographical localization, the type of organization they belong to, their domain of activity, as well as their intended use. This analysis might reflect the concerted worldwide actions or individual initiatives, as well as highlight the most urgent tools needed to face an emergence.

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A Zika virus research toolbox for ZIKAlliance & beyond

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The EU-funded ZIKAlliance consortium, which includes over 53 partner institutions from around the globe, was initiated in response to the ZIKV outbreak in the Americas, and studies a wide variety of aspects of the ZIKV outbreak. ZIKAlliance work includes basic research into the replication and pathogenesis of the virus and the identification of antiviral molecules. This required the development of a collection of ZIKV research tools, like specific (monoclonal and camelid) antibodies, cDNA clones, purified proteins, enzymatic and cell-based assays. Fruitful collaborations and the efforts of a diverse group of ZIKAlliance partners has led to a continuously growing collection of ZIKV research tools and materials. These are not only crucial for basic research into ZIKV replication and antivirals, but could also be useful for other areas of ZIKV research, like diagnostics, surveillance, epidemiology, virus evolution, entomology etc. A number of the available tools will be highlighted. For example, various ZIKV reverse genetics systems and their applications, useful antibodies and a comparison and characterization of ZIKV strains will be discussed.

*Speaker

Zika control in an ethical & legal context: The importance of fast data-sharing and the barriers hampering it

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Genetic information of pathogens is an essential input for infectious disease control, public health and for research. Efficiency in preventing and responding to global outbreaks, such as the one of ZIKAV, relies on timely access to physical samples and genetic sequence data from pathogens. Still, barriers stand in the way of timely sharing of genetic resources from pathogens, frustrating collaborative research, efficient public health responses and ultimately the potential use of such resources in the development of medical countermeasures. Most (re)emerging epidemics rely on the interface between animal, human and environmental health. Under this One Health approach, stakeholders and their interests are manifold and need to be investigated. In order to establish efficient sharing systems, the engagement and support from the different stakeholder groups are essential. Yet, such wide engagement will not be sustained unless stakeholders' interests are attained and pondered against possible concerns. The results of a research based on interviews with key actors from governmental and non-governmental bodies will be presented, in which we identified overlapping and conflicting interests, and the overall challenges for sharing pathogen data, focusing especially on barriers faced by research institutes and international research collaborations. The aim is to create awareness about existing challenges and to provide essential inputs to the discussion about political and practical strategies for improved data sharing practices.

*Speaker

Basic research and antivirals

Posters

Development of a nanobody-based NS1 antigen–capture assay for the specific diagnosis of acute Zika virus infection

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Zika virus (ZIKV) is a mosquito-borne flavivirus that emerged in Brazil in 2015 and then rapidly spread throughout the tropical and subtropical Americas.
Based on clinical criteria alone, ZIKV infection cannot be reliably distinguished from infections with other pathogens that cause an undifferentiated systemic febrile illness, including infections with two common arboviruses, dengue virus (DENV) and chikungunya virus. Diagnostics tools are thus needed to identify patients infected with ZIKV. The choice of a diagnostic method depends on the period between onset of symptoms and sample collection. From the onset of clinical symptoms (Day 0) until the fifth/seventh day of illness (D5-7), diagnosis of flavivirus infections can be done by RT-PCR to detect the viral RNA.

For DENV, detection of the viral NS1 antigen over the same window (D0 to D5-7) has become the reference diagnostic assay over the past few years. The NS1 antigen has also been detected in patients infected with yellow fever (YF) or West Nile (WN) viruses, suggesting that NS1 secretion is a hallmark of flavivirus infections. For ZIKV, the use of NS1 detection for diagnostics needs to be validated.
Camelids have been immunized with a recombinant form of the ZIKV NS1 protein to generate VHHs or nanobodies. These nanobodies were selected for their specificity to ZIKV NS1 and showed no cross reactivity with NS1 from different flaviviruses including DENV, WNV, YFV, Tick-borne encephalitis virus and Japanese encephalitis virus. An ELISA was developed using a broadly cross-reactive mAb for NS1 capture and specific nanobodies against ZIKV NS1 for its detection. This ELISA will be used to specifically detect NS1 in blood of patients and assess the value of this approach as a first intent diagnosis of clinical ZIKV infections.

*Speaker

Therapeutic Treatment of Zika Virus Infection Using a Brain-Penetrating Antiviral Peptide

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Introduction: *Zika virus* (ZIKV) is a mosquito-borne virus that is associated with neurodegenerative diseases, including Guillain–Barré syndrome and microcephaly.
Objective: As ZIKV targets the nervous system; there is an urgent need to develop therapeutic strategies that inhibit ZIKV infection in the brain.

Methods and results: To approach this challenge, we devised an antiviral strategy termed Lipid Envelope Antiviral Disruption, or LEAD. We have performed a detailed characterization of membrane-peptide interactions and validated the targeting range of the stabilized peptide against spherical, membrane-enclosed liposomal nanoparticles, which led us to evaluate antiviral activity across multiple scales spanning from *in vitro* virus neutralization to *in vivo* therapeutic efficacy to *in vivo* brain delivery and protection. We have shown that this brain-penetrating peptide, called AH, exhibits inhibitory activity *in vitro* against ZIKV and other mosquito-borne viruses such as *Yellow Fever virus* (YFV), *Dengue virus* (DENV), *Japanese encephalitis virus* (JEV) and *Chikungunya virus* (CHIKV). More specifically, we investigated whether AH peptide can inhibit ZIKV-induced neuronal death in primary neuronal cultures. Two ZIKV strains, the MR766 African origin and a contemporary ZIKV strain (HS-2015-BA-01) were tested. Cell viability was assessed after 48 hours of ZIKV (or mock) infection, and AH peptide treatment protected against virus-induced cell death. Next, we evaluated the therapeutic efficacy of the AH peptide in a lethal ZIKV mouse model, previously established by our group, with treatment starting three days after infection. Therapeutic treatment protected against mortality and markedly reduced clinical symptoms such as body weight loss and increase of intraocular pressure, viral loads (serum, spleen, brain and optical nerve) and neuroinflammation (by reduction of production of inflammatory mediators in the brain as assessed by levels of IL-1b, TNF-a, CCL5 and CXCL-1 and neutrophil recruitment by dampening mieloperoxidase activity in this organ), as well as mitigated microgliosis, neurodegeneration and brain damage. In addition to controlling systemic infection, the peptide crossed the blood-brain barrier (BBB) to reduce viral loads in the brain, and protected against ZIKV-induced BBB injury.

*Speaker

Discussion/conclusion: Collectively, our findings demonstrate that a brain-penetrating peptide therapeutically inhibits ZIKV infection and neurodegenerative disease, and support its potential for treating neurotropic viral infections.

The SUMOlysation of ZIKA nonstructural protein 5 (NS5) influences its stability, its cellular localization and its interferon blocking activity

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The factors that contributed to the emergence, spread and change in pathogenesis of ZIKV are not fully understood. Using sequence alignments of different ZIKV strains of a range of spatiotemporal history of isolation and representing African and Asian lineages, we identified possible differences in Lysine presence or position. Lysine are the target of important post translational modification (PTM) like Ubiquitination, acetylation or Sumoylation. Recently, we and others demonstrated that the attachment of small ubiquitin-like modifier (SUMO) to some viral protein are important at different stages of the viral cycle. In order to identify potential SUMOylation, we expressed individually all ZIKV proteins in the presence of tagged SUMO and different proteins of this pathway. We identified NS5 as the sole ZIKV protein being SUMOylated in a classic mechanism, as recently illustrated with the DENV NS5 protein. Using RNA interference strategy, we found that changes in the SUMO cell machinery inhibit Zika replication. Using sequence alignments and site directed mutagenesis, we identified a SUMO interacting motif (SIM), well conserved among Flaviviruses. that is responsible for a non-covalent binding of SUMO protein in both African and Asian/American strains. Different NS5 SIM mutants that we generated showed both no SUMOlysation and a severe defect in viral RNA replication. In addition, we found that the SUMOylation of NS5 regulates its ability to localize in punctuated structures in nuclei similar to PML nuclear bodies. Finally, SUMOylation-defective mutants failed to degrade STAT2 and cannot counteract host antiviral interferon signaling. This is the first report showing that ZIKV NS5 SUMOlysation is crucial for viral replication. Nevertheless, the role of NS5 Lysine motifs in ZIKV virulence remains to be investigated. The SUMO cell machinery represents a possible target for drug development against ZIKV.

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Japanese encephalitis virus-specific CD8 T-cells cross-react with HLA-A2-restricted Zika virus epitopes

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Zika virus (ZIKV) has become a global health threat due to association with severe congenital malformations and its widespread transmission1. Nevertheless, its spreading is really limited in China and South-East Asia despite the presence of ZIKV transmission-competent mosquitoes, and the circulation of other flaviviruses including Dengue virus (DV), Japanese encephalitis virus (JEV) and Chikungunya virus2. Several hypotheses have been suggested, including cross-protective immunity provided by other endemic flaviviruses. While cross-reactivity of DV immunity with ZIKV infection have been reported because of the presence of DV in South America3, little is known about ZIKV-JEV immunologic cross-reactivity, and whether JEV pre-existing immunity may provide protection or contribute to ZIKV pathogenesis.
As pre-exposure to JEV or JEV-vaccine elicits cross-reactive CD8 T cell immunity in humans against DV infection4, we were wondering if exposure to JEV or JEV-vaccine would elicit cross-reactive CD8 T cell immunity to ZIKV infection. To answer this question, we used epitope prediction algorithms and HLA-A2 transgenic mice5 to investigate the immune cross-reactivity of ZIKV and JEV.

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In vitro model for flavivirus oronasal transmission using apical infection of porcine and human respiratory epithelial cells in air-liquid interface cultures

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Flavivirus represent an important public health threat with a worldwide distribution. Some of those zoonotic viruses, as JEV, WNV or ZIKV, are neurotropic being responsible of important central nervous system diseases as encephalitis or microcephaly. Flaviviruses are mosquito-transmitted and kept in enzootic cycle with different vertebrates acting as amplifying host, such as pig for JEV. Recently we have observed efficient oronasal JEV transmission by contact resulting in efficient infection as well as oronasal virus shedding. In vaccinated animals we also observed oronasal virus shedding in absence of viremia. Moreover, a ZIKV direct transmission between guinea pigs through oronasal contact has been recently published. Those findings suggest that flaviviruses are able to infect cells from the respiratory mucosa. In this work, we demonstrated JEV infection and replication in porcine nasal epithelium of cultured explants. In addition, air-liquid interface cultures of nasal epithelium were susceptible to JEV genotype 1 and 3 strains. Viral shedding at was found between 24 and 72 hours post-infection, at both apical and basal sides of the cultures. Infected cells did not stimulate antiviral and pro-inflammatory cytokines. We also found that JEV infection induced an increase of epithelial cell death, and induction of chemokines attracting monocytes. Flavivirus infection of human nasal epithelial cells was donor dependent. While all donors' cells were infected by JEV, the susceptibility of infection by WNV, ZIKV or USUV varied between donors. These results demonstrate that nasal epithelial cells are susceptible to flavivirus and could play an important role in non-vector mediated viral transmission occasionally observed in vertebrates. This may have implications for biosafety of laboratory personnel, healthcare providers and animal caretakers.

*Speaker

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Functional characterization of the RNA-dependent RNA polymerases (RdRp) of dengue (DENV) and Zika virus (ZIKV)

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The function of the RNA-dependent RNA polymerase (RdRp) of flaviviruses (single-strand positive-sense RNA viruses, family Flaviviridae, genus Flavivirus) is harbored in the C-terminal RdRp domain (NS5pol) of non-structural protein NS5. The RdRp assures the replication of the viral genome and is therefore essential. The N-terminal domain bears several functions implicated in the formation and methylation of the RNA cap of the genomic RNA. The latter serves directly after infection as messenger RNA. The RdRp starts RNA synthesis de novo, i.e. it produces its own primer during the so-called initiation phase before entering the elongation phase. We have shown that 1) de novo initiation of the RdRp domain of DENV serotype 2 (DENV2) NS5 is using a priming loop and more specifically a His residue strictly conserved in the Flavivirus genus; 2) the RdRp domain shows a pronounced preference to start initiation with an A, proposing the existence of a specific A initiation site; 3) after the synthesis of the primer pppAG (or pppAGA) the RdRp changes to the processive elongation phase; 4) the N-terminal domain of DENV2 NS5 stimulates the initiation and elongation phase of the RdRp and 5) the elongation complexes of DENV2 and ZIKV NS5 with specific primer/templates incorporate to a very low extent 2'-C-Me-2'-F nucleotides similar to Sofosbuvir, an antiviral drug currently in clinical use against hepatitis C virus (family Flaviviridae, genus Hepacivirus).

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Multiple strategies to identify new Zika virus inhibitors

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With the aim of identifying novel compounds endowed with antiviral activity against Zika virus (ZIKV), multiple approaches spanning experimental and computational studies have been performed. The cell-based screening of our in-house library revealed that a series of antivirals was also effective against ZIKV with low micromolar activity and a good safety profile. Derivatives of the same series have been synthesized with the aim of increasing the antiviral activity. In order to discover new series of ZIKV inhibitors, a virtual screening (VS) study combining molecular dynamic (MD) simulations, pharmacophore screening and consensus docking was performed for the identification of new small-molecule inhibitors of ZIKV nonstructural protein 5 methyltransferase (NS5 MTase), which is essential for flaviviral viral replication.[1] Indeed, the NS5 MTase methylates the cap structure of viral RNA at N7 and 2'O position. The N7 methylation is essential for viral RNA translation into proteins and the 2'O methylation limit viral detection by the innate immunity. Few top-scored compounds that were supposed to bind ZIKV NS5 MTase within the binding site for its substrate S-Adenosyl methionine were purchased and subjected to enzymatic assays. Among these, compound GP1 showed inhibitory activity on ZIKV and dengue virus NS5 MTase with IC50 values of 34 μM and 42 μM, respectively.

Finally, a ligand-based similarity search performed on our in-house library was carried out to select compounds structurally similar to seliciclib, a CDK kinase inhibitor that showed to inhibit ZIKV production in glioblastoma SNB-19 cells with nanomolar potency.[2] Among the representative compounds that were tested in ZIKV-infected Vero cells, GSC-3 and GSC-8 showed the most interesting activity, with EC50 values around 2 μM and CC50 values around 17 μM. Overall, our multifaceted research approach allowed the identification of new ZIKV inhibitors belonging to different chemical series, which represent valuable starting points for the development of high potent antiviral compounds.

*Speaker

Zika tropism for different cell populations migrating into the developing cerebral cortex

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The outbreak of Zika virus in Brazil in 2015 has raised strong worldwide interest due to its vertical transmission from the infected mothers to their foetuses. Indeed, the percentage of infected newborns that exhibited signs of microcephaly and other neurodevelopmental defects was remarkably increased compared to the average number of cases in non-infected individuals. Furthermore, clinical studies have highlighted a strong correlation between Zika infection and neonatal abnormalities, while successive research on cell and animal models have confirmed the causal link.

Recent studies have ascertained the tropism of Zika for many different types of cells: from microvascular endothelial cells to different types of placental cells (Trophoblasts, Hofbauer cells, etc.) from Leydig or Sertoli cells (testis) to the brain neuronal progenitors as well as different types of eye cells (e.g. ganglionic and bipolar cells). However, it is still unclear how the virus reaches and crosses the placental barrier, and how it subsequently disseminates to different foetal organs.

Our study aims at identifying cellular vectors that transport the Zika virus from the placental tissue to the embryonic brain and/or spread the infection into the developing cerebral cortex. We test Zika tropism for subgroups of cell populations born in extra-embryonic or extra-cephalic territories, which begin to invade the brain at early stages of development, as well as for neuronal or non-neuronal cells migrating to the cerebral cortex from different regions of the telencephalon. To investigate their possible role in trafficking Zika virus, we are infecting transgenic mouse lines with fluorescent reporters for distinct cell populations, as well as applying genetic and chemical tools to specifically impair their migration and/or maturation of these cells.

*Speaker

Development of Zika virus neutralisation assays in a neuronal cell line.

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Zika virus (ZIKV) is a positive sense single stranded RNA virus, belonging to the *Flaviviridae* family which includes numerous pathogens of medical importance including dengue virus, West Nile Virus and tick-borne encephalitis virus. The sudden and sporadic outbreak of Zika virus (ZIKV) in the Yap Islands in 2007 followed by outbreaks in other Pacific islands, Southeast Asia and Latin America, most notably the 2015-16 epidemic in Brazil, has focussed international attention on ZIKV. Furthermore, the link between ZIKV and the development of fetal and congenital abnormalities, such as microcephaly, has spurred scientific research. The demand for a safe and effective vaccine requires novel assays that quantify ZIKV infection and neutralisation. The aim of this work is to develop flow cytometry based neutralisation assays as an alternative to plaque reduction neutralisation tests (PRNT's), and also comparison of ZIKV neutralisation in two different cell lines: 1) Vero cells, African Green monkey kidney epithelial cells, and 2) SH-SY5Y, a neuronal-like cell line. Establishment of flow cytometry based neutralisation assays using a neuronal-like cell line may allow for more representative assays and enable identification of more specific receptors used by the ZIKV for neuronal infection. Future work aims to investigate ZIKV infection in human embryonic stem cells, specifically analysing infection during stages of neuronal differentiation.

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Structure-based design of a Zika virus diagnostic antigen

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The Latin America and the Caribean are currently facing an epidemic of Zika virus (ZIKV) infection with unprecedented reports of neurological sequelae with profound impact in the economy and health systems in the affected areas. The need of effective prophylactic measures as well as diagnostic tools to stop the disease spread are still unmet public health priorities. The current diagnostic algorithms to identify ZIKV infection still rely on the severity of clinical symptoms and viral detection through molecular techniques. However, differentiation between ZIKV and other related Flavivirus infections is a hard task due to the large overlap and well-described cross-reactivity between members of the Flavivirus family in serological assays. We hypothesize that effective diagnostic antigens should display the native- like structure of viral epitopes as they are recognized by human antibodies. To this aim, B cell epitope prediction tools were used to investigate amino acid sequences within the envelope (E) ZIKV protein for antibody binding. The E protein is well know as main target of humoral response in the context of a natural infection by Flavivirus and is considered the best candidate for vaccine and diagnostic approaches. A 9 residues sequence was identified and the corresponding structural motif was computationally grafted into a scaffold protein named Top7. Multiple rounds of combinatorial mutations on the scaffold were performed to ensure that the epitope is displayed in its native-like conformation and the stability and solubility of the scaffold. Molecular dynamics simulations were used to confirm the thermodynamic and structural stability of the designed protein. ELISA assays were used to assess the maintenance of the immunoreactivity of the ZIKV epitope within the Top7 scaffold showing that the chimeric protein is properly recognized by specific IgG antibodies in the serum of ZIKV infected individuals. Further analysis comparing sera samples from ZIKV+ DENV- and ZIKV- DENV+ individuals demonstrated that the developed chimera exhibits 75% sensibility and 50% specificity. Amino acid sequences analysis revealed that, although the grafted sequence does not show sequence identity with any other E sequences from DENV, the sequence exhibits over 70% identity with NS2b/NS3 of DENV. Even though the diagnostic performed of chimera is not sufficient to differentiate between ZIKV and DENV, our results are encouraging as the developed protocol might be used as a platform to engineer antigens with improved biological functions aimed to be used as vaccine or diagnostic tools in future approaches.

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The SUMOlysation of ZIKA nonstructural protein 5 (NS5) influences its stability, its cellular localization and its interferon blocking activity

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The factors that contributed to the emergence, spread and change in pathogenesis of ZIKV are not fully understood. Using sequence alignments of different ZIKV strains of a range of spatiotemporal history of isolation and representing African and Asian lineages, we identified possible differences in Lysine presence or position. Lysine are the target of important post translational modification (PTM) like Ubiquitination, acetylation or Sumoylation. Recently, we and others demonstrated that the attachment of small ubiquitin-like modifier (SUMO) to some viral protein are important at different stages of the viral cycle. In order to identify potential SUMOylation, we expressed individually all ZIKV proteins in the presence of tagged SUMO and different proteins of this pathway. We identified NS5 as the sole ZIKV protein being SUMOylated in a classic mechanism, as recently illustrated with the DENV NS5 protein. Using RNA interference strategy, we found that changes in the SUMO cell machinery inhibit Zika replication. Using sequence alignments and site directed mutagenesis, we identified a SUMO interacting motif (SIM), well conserved among Flaviviruses. that is responsible for a non-covalent binding of SUMO protein in both African and Asian/American strains. Different NS5 SIM mutants that we generated showed both no SUMOlysation and a severe defect in viral RNA replication. In addition, we found that the SUMOylation of NS5 regulates its ability to localize in punctuated structures in nuclei similar to PML nuclear bodies. Finally, SUMOylation-defective mutants failed to degrade STAT2 and cannot counteract host antiviral interferon signaling. This is the first report showing that ZIKV NS5 SUMOlysation is crucial for viral replication. Nevertheless, the role of NS5 Lysine motifs in ZIKV virulence remains to be investigated. The SUMO cell machinery represents a possible target for drug development against ZIKV.

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The European Virus Archive resource for Zika virus

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The "European Virus Archive" (EVA) is a collection of viruses and virus-derived products that are accessible on a non-profit basis for academic researchers.

EVA collection is managed by the EVA consortium (EVAg) including 24 partners worldwide. The objectives are to develop and maintain a large updated virology resource in order to promote access for academics and industrials. In addition, the role of EVAg is also to mobilize specific task force in case of emergence in order to support public health response, as exemplified with the Zika situation.

A total of 1300 viruses and 800 virus-derived products are accessible in the online catalog at *https://www.european-virus-archive.com/evag-portal.*

- For Zika virus (ZIKV), 19 viral strains and 35 ZIKV-derived products are in the catalog. They possess high standards of quality (complete sequence, titrated material): a large variety of products is available: virus strains, recombinant proteins, viral RNA, cDNA, inactivated lyophilized titrated samples for quality control in the field of diagnosis.

- EVA collection has and continue to support the international response during the ZIKV outbreak: more than 300 ZIKV related products were supplied worldwide.

- EVA catalog includes
  (i) relevant panel of ZIKV clinical strains
  (ii) molecular ZIKV diagnostic tools incl. ready to use diagnosis kits (WHO recommended)
  (iii) synthetic encapsulated positive controls and external quality control
  (iv) non-infectious quantitated lyophilised samples (sera, urine ...) for quality assessment
  (v) and research tools such as reverse genetic systems and expression plasmids

- Academics can access EVA material "free of charge" (only shipping fees are due) after validation by the Selection panel consisting of researchers.

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- EVA collection is providing high-quality resources for research programmes funded by the European Commission such as ZikAlliance, EVD-LabNet, EbolaModRad, Silver etc....

ISA reverse genetics method for emerging RNA viruses: 1. Original description

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Reverse genetics is a key methodology for producing genetically modified RNA viruses and deciphering cellular and viral biological properties, but methods based on the preparation of plasmid-based complete viral genomes are laborious and unpredictable. Here, both wild-type and genetically modified infectious RNA viruses were generated in days using the newly described ISA (infectious-subgenomic-amplicons) method. This new versatile and simple procedure may enhance our capacity to obtain infectious RNA viruses from PCR-amplified genetic material.

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ISA reverse genetics method for emerging RNA viruses: 2. Technical improvements and ISA derived-methods

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The ISA (infectious subgenomic amplicons) method is a new bacterium-free reverse genetics method that has been successfully applied to a variety of single-stranded positive-sense RNA viruses. Here, we describe three technical improvements of this method. The first, named ISA-lation, was used to rescue infectious virus from non-infectious clinical and/or animal samples. The second, named Haiku, is an ultimately simplified design in which terminal pCMV and HDR/SV40pA sequences are provided as additional separate DNA amplicons. The third is an improved DNA transfection protocol to generate infectious viruses in insect
cells. We also developed an ISA-derived genetics method, named SuPReMe (Subgenomic Plasmids Recombination Method), which give the possibility to rapidly generate clonal population of recombinant viruses.

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ISA reverse genetics method for emerging RNA viruses: 3. Comparison of genotypic and phenotypic characteristics of Chikungunya viruses generated using the ISA method or classical infectious clone technology

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Reverse genetics systems provide the opportunity to manipulate viral genomes and have been widely used to study RNA viruses. Several reverse genetics procedures are now available to produce wild-type and genetically modified viruses, and their impact on genotypic and phenotypic characteristics of the viruses is unknown. Here, by exploiting a chikungunya virus model, we compare *in vitro* and *in vivo* using *Aedes* mosquitoes the genotype and the phenotype of viruses generated using either the rapid and user-friendly ISA (Infectious Subgenomic Amplicons) method or classical infectious clone technology. Our results demonstrated that the ISA method led to a higher genetic diversity of viral populations, but no genotype difference was observed.

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ISA reverse genetics method for emerging RNA viruses: 4. Reverse genetics platform to study Zika virus

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Zika virus (ZIKV) is a typical example of a re-emerging pathogen. Recently, ZIKV invaded the American continent with large outbreaks occurring in many territories of South and Central America and associated with congenital diseases and neurological complications. Deciphering the natural history, ecology and pathophysiology of this mosquito-borne pathogen requires effective reverse genetics tools. Thus, we generated four simple and performing reverse genetics systems for relevant ZIKV strains that are representative of the ZIKV genetic diversity. Two were designed directly from viral genome databases: one is based on a low-passaged ZIKV African strain (Dakar 1984) and the second is based on an old Asian strain (Malaysia 1966). The other two systems were based on recent clinical ZIKV strains (French Polynesia 2013 and Martinique 2015). All systems allowed to rescue wild-type and a variety of specifically engineered chimeric ZIKVs in days using the user-friendly PCR-based ISA (Infectious Subgenomic Amplicons) method and a recently ISA-based reverse genetics method, named SuPReMe (Subgenomic Plasmids Recombination Method), that allows to generate clonal population of viruses.

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Basic research and antivirals

Oral presentations

Tomatidine, a novel antiviral compound against dengue & chikungunya virus

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Dengue virus (DENV) and Chikungunya virus (CHIKV) cause debilitating diseases mostly in the (sub)tropical regions of the world. Dengue is the most common arthropod-borne infectious viral disease with an estimated 400 million infections annually. Despite its major impact on global human health and huge economic burden there is no antiviral drug available to treat the disease caused by these viruses. In this study, we describe tomatidine as a novel compound with potent antiviral properties towards DENV and CHIKV. The EC50 and EC90 values are within the sub-μM range following infection of Huh7 cells at MOI 1. The production of infectious virus particles was equally reduced to that of the production of genome equivalent particles. No antiviral activity was seen for West Nile virus and Zika virus. Subsequent time-of-drug-addition experiments revealed that tomatidine acts on multiple stages of the viral life cycle yet different results were obtained for CHIKV and ZIKV. During the conference we will share our knowledge regarding the antiviral properties of tomatidine towards DENV and CHIKV.

*Speaker

ZIKV infection disregulates neurogenesis through the Notch pathway in human neural progenitor cells

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Zika virus (ZIKV) is a mosquito-borne Flavivirus that causes Zika disease with particular concerns regarding neurological complications, such as Guillain-Barre Syndrome and congenital microcephaly. ZIKV targets human brain cells, reducing their viability and growth as neurospheres and brain organoids suggesting that ZIKV could affect human brain development by abrogating neurogenesis. ZIKV has shown to directly infect human cortical neural progenitor cells (hNPc), causing transcriptional dysregulation, and attenuating cell growth. Nevertheless, it is unknown which of the cellular pathways involved in the disruption of hNPC neurogenesis are targeted by ZIKV infection. Therefore, our study compares the effect of two ZIKV strains on the differentiation process of hNPC with the aim to provide a comprehensive view of the signaling pathways that mediate neural tissue depletion following viral infection.
The susceptibility of hNPc to infection of two strains of ZIKV (African and Asian lineages) was evaluated according to the cellular differentiation state. The expression of ZIKV-induced antiviral innate immune response genes in proliferating and/or differentiated hNPc was determined by qPCR and Western Blot analysis. The effect of ZIKV infection during hNPC differentiation was explored by screening the neurogenesis gene expression profile using qPCR array and cell type characterization using immunofluorescence techniques.

Our results showed that the differentiation process of hNPc led to a decreasing susceptibility to infection with each of the ZIKV strains. Undifferentiated hNPc are not only highly permissive to ZIKV infection, but infection, particularly with the african strain, is also associated with increased cell death. These results could be explained by a stronger and earlier antiviral innate immune response observed in infected differentiated hNPc as compared to undifferentiated cells. Our data also demonstrated that ZIKV modulâtes the expression profile of genes in hNPC, important in neurogenesis through the modulation of the Notch pathway which is involved in cellular proliferation, apoptosis and differentiation.

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These results show that the differentiation state of hNPc is a significant factor for the outcome of ZIKV infection which is associated with immune response maturity. Our study suggests that ZIKV infection may initiate early activation of the Notch pathway resulting in an abnormal differentiation process that may be implicated in ZIKV-induced brain injury.

ZIKA virus replication in human placenta

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Zika virus (ZIKV) is transmitted vertically and id associated with severe fetopathy, including microcephaly. To decipher the mechanisms involved in ZIKV transmission from mother to fetus, we have studied the susceptibility of human placental cells and explants to the virus. The human trophoblastcell line Jeg-3 was shown to be susceptible to ZIKV infection, but resistant to Chikungunya virus (CHIKV) infection, another emerging arbovirus which has been shown to be unable to infect the
placenta and be exclusively transmitted vertically from viremic mother during parturition via placental breaches. We infected third trimester human placental explants with either ZIKV or CHIKV, and determined viral load in placental tissues and supernatants daily from day 1 to day 4 post-infection. While ZIKV replicates in human placental explants, in contrast to CHIKV. Moreover, we show that infection in the presence of low concentration of antibodies to ZIKV increases infection of explants.
Whether antibodies increase infection by increasing ZIKV ability to cross the placental barrier is being investigated. In human explants, we identified Hobauer cells as target cells of ZIKV, which corroborates data obtained by other groups. We are currently studying target cells of ZIKV in placental biopsies of infected pregnant women, and this will allow us to investigate the relevance of our experimental findings in human. Understanding the placental phase of ZIKV infection is required to understand its vertical transmission and develop potential preventive and therapeutic strategies against this emerging
teratogenic virus.

*Speaker

Stress-induced unfolded protein response contributes to Zika virus-associated microcephaly

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Accumulating evidence support a causal link between Zika virus (ZIKV) infection during pregnancy and congenital microcephaly. However, the mechanism of ZIKV-associated microcephaly remains unclear. We combined analyses of ZIKV-infected human fetuses and cultured human neural stem cells with mouse embryos to understand how ZIKV induces microcephaly. After intracerebral and intraplacental inoculation of ZIKV in mouse embryos, we show that it triggers endoplasmic reticulum stress in embryonic brains *in vivo*. This perturbs a physiological unfolded protein response within cortical progenitors that controls neurogenesis. Thus, ZIKV-infected progenitors generate fewer projection neurons that eventually settle in the cerebral cortex whereupon sustained ER stress leads to apoptotosis. Furthermore, we demonstrate that administration of pharmacological inhibitors of UPR counteracts these pathophysiological mechanisms, and prevents microcephaly in ZIKV-infected mouse embryos. Such defects are specific to ZIKV as they were not observed upon intraplacental injection of other related flaviviruses in mice.

*Speaker

ZIKV NS5: target-based drug development

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[2] Université Montpellier 2 – CNRS : UMR5247 – France
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Zika NS5 is a bi-functional enzyme ensuring RNA genome synthesis as well as RNA capping. It is a validated target for drug-design since i) it is essential for virus growth, i) it is the most conserved Flavivirus protein, and iii) a drug could be highly selective, since NS5 has no structural nor functional equivalent in the infected cell. The RNA-cap Mtase, the Polymerase, and the way they talk to each other through their protein-protein interface constitute well defined targets. I will describe the current knowledge on the mechanisms of both enzymes, as well as the substrate specificities during the Flavivirus life-cycle. For the Mtase, how a guanosine could be N7-methylated, then a 2'-O methyl added to the first transcribed adenosine, as well as how internal methylation may occur, and its potential roles. For the polymerase, I will present which residues in conserved motifs A-G may participate in RNA synthesis fidelity, with connection to the current knowledge on Picornavirus ad HCV polymerases, and in the context of the design of antiviral drugs.

*Speaker

Therapeutics for Microcephaly

Ivan Gladwyn-Ng *† [1], Christian Alfano [1], Lluis Cordon-Barris [1], Catherine Creppe [1], Thérèse Couderc [2], Marc Lecuit [2,3], Laurent Nguyen [1]

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[3] Institut Imagine, Department of Infectious Diseases and Tropical Medicine – Université Paris Descartes, CHU Necker - Enfants Malades [AP-HP] – France

While the causal link between Zika virus (ZIKV) infection during gestation and congenital microcephaly is increasingly supported by recent publications, the underlying mechanisms remain poorly elucidated. Our recent publication showed that ZIKV triggers endoplasmic reticulum (ER) stress in the cerebral cortex through combinatorial analyses of ZIKV-infected post-mortem human foetuses, mouse embryos and cultured human neural stem cells. The virus perturbs a physiological unfolded protein response (UPR) within cortical progenitors that controls neurogenesis, which leads to diminished generation of projection neurons.
Here, we demonstrate, via cellular and genetic assays, that intracerebral administration of different pharmacological inhibitors of distinct arms of the UPR counteracts the pathophysiological up-regulation of the ER stress within ZIKV-infected mouse embryonic brains. Concomitantly, the pharmacological inhibition of ZIKV-induced ER stress / UPR by different compounds was associated with the prevention of microcephaly in infected embryonic mice.
In conclusion, we developed an *in vivo* model for ZIKV infection, as well as validated it for the testing of potential pharmacological compounds. Our results suggest the pharmacological inhibition of ER stress-induced UPR may contribute towards a treatment strategy in ZIKV congenital infections.

*Speaker
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Development of highly potent Zika/flavivirus inhibitors; lessons from HCV drug development

Johan Neyts *† [1]

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The zika and other flaviviruses belong with the hepatitis C virus (HCV) to the family of the Flaviviruses. In recent years highly potent inhibitors of the HCV have been developed; these include inhibitors of the RNA dependent RNA polymerase (RdRp), NS3 protease inhibitors as well as compounds that target NS5A, an essential viral protein without enzymatic activity. Combinations of such drugs (that are very well tolerated) result within 8 to 12 weeks in a cure of $> 95\%$ of patients with chronic HCV, even if in relatively advanced stages of the diseases. It should hence well be possible to develop also highly potent inhibitors of the Zika and other flaviviruses. Like HCV, flaviviruses encode for a RdRp and a NS3 protease but not for NS5a. However, we and others have demonstrated that NS4b of flaviviruses, is an excellent target for inhibition of flavivirus replication. Besides the Zika virus, many other flaviviruses pose a serious treat to human health and new flaviviruses may possibly emerge in the future. Hence, rather than having virus-specific antivirals, it may be of value to have pan-flavivirus compounds at hand. I will explain our efforts in developing dengue, zika and pan-flavivirus inhibitors.

www.antivirals.be
www.facebook.com/NeytsLab

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Zika vaccine

Posters

Establishment of the WHO 1st International Standard for Zika antibody: results of multi-centre collaborative study

Mark Page * [1], Giada Mattiuzzo [1], James Ashall [2], Sophie Myhill [1], Valwynne Faulkner [3], Mark Hassall [1], Dianna Wilkinson [1], Peter Rigsby [1], Jason Hockley [1], Eleanor Atkinson [1], Stacey Efstathiou [1]

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Accurate diagnosis in an outbreak scenario is crucial for the provision of appropriate healthcare and treatments. For emerging pathogens, the assays used for the diagnosis are either absent or poorly developed. Their production and approval need to be rapid while still assuring the quality of such assays. A vital tool is the provision of appropriate reference standards. We collected serum and plasma samples from Zika exposed individuals and confirmed by in house assays the presence of anti-Zika antibodies. Collaborative study samples also included a range of negative sera/plasma, human anti-Zika IgG from immunised trans-chromosomal cows and Dengue antibody reagents. Freeze-dried preparations of the human sera/plasma were distributed to 19 laboratories in 6 countries which then tested them with a range of ELISAs and virus neutralisation assays. The data were analysed to identify a suitable candidate material that would serve as a calibration standard.
There was good concordance across most assays, with similar ranking of the samples based on antibody titre. An outlier in this trend was observed when an African lineage virus was used in the neutralisation assay. Neutralisation assays using reporter viruses also gave concordant results. Qualitative ELISAs showed that the convalescent sera/plasma contain both anti-Zika IgG and IgM.
The results of the study support the proposal of a serum pool as the 1st WHO International Standard which will be submitted to the WHO Expert Committee for Biological Standardisation in October 2018.

*Speaker

Zika vaccine

Oral presentations

First generation MV-Zika vaccine – fast track clinical development

Sabrina Schrauf * [1]

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Themis' is developing a safe, effective and affordable preventive vaccine platform against priority pathogen diseases that have the potential to cause epidemics such as Chikungunya or Zika virus infection by using a "plug-and play" vaccine technology. This technology is based on a measles vaccine vector (MV) that can be easily genetically modified to express immunoprotective proteins for designated emerging infectious pathogens. This delivery platform technology has already demonstrated proof of principle in humans through a Phase 1 clinical trial with a recombinant measles vaccine against Chikungunya virus (MV-CHIK). We were now able to demonstrate vaccine safety and immunogenicity in 260 subjects in an ongoing Phase 2 clinical trial (interim analysis). In addition, we are able to demonstate that the measles based vaccine's potency is not impaired in the presence of pre-exisiting anti-measles immunity. In 2015, Zika virus spread in the Americas and to date caused autochthonous, vector-borne transmission in 84 countries and territories worldwide. This rapid emergence of the previously unknown pathogen raised the urgent need for a vaccine that can be rapidly produced in response to a newly emerging pathogen. Themis took the challenge and developed a vaccine candidate from design to Phase 1 clinical trial submission within 11 months. The clinical trial is ongoing and final data will be available later this year.

*Speaker

Phase II/III clinical trials: endpoints, clinical trial sites/(target) study population & ethical considerations

Thomas Jaenisch * [1]

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Once the first ZIKV vaccine candidates have passed pre-clinical testing, the clinical trials will have to be designed and initiated. Under the assumption that ZIKV transmission will be further reduced for the coming years, this will be challenging for a number of reasons:
a) Endpoints: A possible endpoint would be virologically confirmed ZIKV infection because of the fact that abnormalities are much less frequent, and because abnormalities have been reported to show considerable variability between countries and regions, which is still not well understood. However, ZIKV infection frequently goes unnoticed and can be asymptomatic in up to 80%. Immunogical endpoints are an alternative, but need to be carefully defined.

b) The possible clinical trial sites for clinical testing need to be selected according to endemicity if infection or abnormality is chosen as an endpoint. This will require both surveillance as well as mathematical modelling due to the rapid dynamic of ZIKV outbreaks.

c) Though the complications of public health concern occur in pregnant women and their unborn children, the target populations could also be the general population or the female population (before becoming pregnant). The question arises if pregnant women should or should not be included, which has implications on the choice of the vaccine candidate (life-attenuated vs. subunit).
Ethical considerations are of relevance for the study design, the selection of sites, and the target populations implicated. The presentation will give an overview about the decisions to be made and ongoing discussions.

*Speaker

A highly efficient and safe yellow fever virus 17D based chimeric Zika virus vaccine candidate

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The recent Zika virus (ZIKV) epidemic in the Americas led to an intense search for therapeutics and vaccines. We here report the engineering of a chimeric vaccine candidate (YF-ZIKprM/E) against Zika by replacing the antigenic surface glycoproteins and the capsid anchor of YFV-17D with those of a prototypic Asian lineage ZIKV isolate that lacks the S139N mutation associated with neurovirulence. The chimeric virus was initially over-attenuated and did not spread in cell culture. By intracellular passaging in dividing cells, an adapted YF-ZIKprM/E virus was obtained that carries mutations in the E gene and 3'UTR. In stark contrast to YFV-17D, YF-ZIKprM/E does not replicate in mosquito cells. Moreover, in AG129 mice (highly susceptible to flaviviruses) and intracranially inoculated Balb/c mouse pups, YF-ZIKprM/E is very safe and at least up to 10ˆ6 fold attenuated over YFV-17D (Stamaril®). A single dose as low as 1x10ˆ2 PFU of the chimeric virus results, as early as 7 days after vaccination, in seroconversion to ZIKV-specific neutralising antibodies and confers full protection against a challenge with a highly lethal inoculum (10ˆ5 LD50) of ZIKV (homologous and heterologous strains). Vaccination results in near sterilising immunity that may be long-lasting. ZIKV structural and YFV-17D non-structural proteins are targets of both CD4+ and CD8+ T cell responses, indicating that also multi-functional (memory) T cell responses may contribute to the protective effect. The particular characteristic of YF-ZIKprM/E in terms of safety and efficacy makes it an excellent ZIKV vaccine candidate.

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Introduction to Zika vaccines and to the ZIKAVAX project

Frédéric Tangy * [1], Etienne Simon-Lorriere [1], Atoussa Arbabian [1], Chantal Combredet [1], Claude Ruffie [1], Romain Marlin [2], Roger Le Grand [2], Sabrina Schrauf [3], Matthias Muellner [3], Katrin Ramsauer [3], Odile Leroy [4], Nicola Viebig [4]

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Over the last two decades a number of new viral diseases such as SARS, MERS or Nipah virus infections have emerged at the animal-human interface, and infections such as Ebola, Lassa, or Zika viruses have expanded beyond their usual limited territories. Each time, the global community failed to develop effective interventions in a timely manner.
Zika virus is an emerging pathogen of substantial public health concern. Although most infections are asymptomatic or benign, a small percentage of patients have complications, such as congenital anomalies in the developing fetus of pregnant women, and neurological complications (Guillain-Barré syndrome). To date, there is no vaccine, antiviral drug, or other modality available to control Zika virus infection.

We will present vaccine development efforts to date with about 30 vaccine candidates in development. We will discuss the immunity to Zika virus and the need for a better understanding to establish correlates of protection. We will discuss how evaluating vaccine immunogenicity and efficacy in healthy adults and in the subpopulations affected (children, pregnant women, women of childbearing age, and elderly people), and the possible effects of Zika vaccination on subsequent dengue virus infection.

The overall objective of ZIKAVAX project is the fast-track development of a safe, effective, and affordable preventive vaccine against Zika virus infection using a delivery platform based on a measles vaccine vector (MV). This platform has demonstrated proof of principle in humans and a preclinical track record of rapid adaptability and effectiveness for a variety of pathogens. Live attenuated measles vaccine is one of the safest and most efficacious vaccines available. Measles vaccinationhas been used for more than 40 years in over 3 billion children and is 95% efficacious after one or two administration. Measles vaccine is genetically stable and reversion to pathogenicity has never been observed. Our ultimate goal is the demonstration of safety and immunogenicity of a recombinant MV-ZIKV vaccine candidate in adult volunteers, including the identification of a suitable immunogenic dose and the demonstration of an acceptable reactogenicity profile. The vaccine will have to induce in volunteers with pre-existing immunity to measles significant levels of Zika virus-specific neutralizing antibodies, and ideally also measur-

*Speaker

able CD4 and CD8-positive cells.
Different recombinant MV-ZIKV vectors have been generated to identify a protective Zika antigen. Preclinical immunogenicity and efficacy studies have been performed in mice that allowed identifying a candidate that elicited strong neutralizing antibodies and complete protection after a single administration. This candidate will be soon introduced in a non-human primate model that we developed. The clinical plan will be presented.

Zika vaccine roadmap: obstacles & opportunities

Annelies Wilder-Smith [*†] [1]

[1] Umeå University – Sweden

At least 45 Zika vaccine candidates have been or are in development, some of them already in phase 2 clinical trials. Both traditional (purified inactivated, live attenuated, recombinant sub-unit) and more novel (DNA, self-replicating RNA, messenger RNA (mRNA), viral-vectored) ZIKV vaccine platforms are in development. Multiple vaccine platforms have shown robust protection against ZIKV challenge in animal models. However, unique challenges will need to be addressed in the clinical development and regulatory pathways of a ZIKV vaccine that may hinder the development, licensure, and WHO-prequalification of high-quality, safe, and effective ZIKV vaccines. Implementing phase 3 efficacy trials will be difficult given the challenges of the spatial and temporal heterogeneity of ZIKV transmission, the broad spectrum of clinical manifestations making a single definite endpoint difficult, the lack of sensitive and specific diagnostic assays, and the need for inclusion of vulnerable target populations. Given the global decline in ZIKV incidence and the potential bottleneck in identifying suitable trial sites, a proposal was made during the June 2017 WHO technical consultation to establish a transparent framework for prioritizing vaccines to be evaluated in phase 2b/3 trials. Selection criteria would depend on the desired attributes, including compliance with the target product profile, pre-clinical evidence of complete or near-complete prevention or reduction of viremia, safety during pregnancy, and scalability of the product.

With the rapid decline in cases, and the still poorly defined use scenarios, the commercial market has become questionable. The prospect of a licensed Zika vaccine is at stake. Mechanisms will be needed to ensure that out of the many Zika vaccine candidates, at least one will make it to the finish line.

The global research and public health community should prioritize the development of ZIKV vaccines that will be acceptable for use by women of reproductive age, and ensure availability and affordability for use in countries where ZIKV is circulating. To this end, WHO is working towards a roadmap for Zika vaccine and product development.

[*]Speaker

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NHP ZIKA infection model

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Animal models of ZIKA virus infection (ZIKV) are crucial to better understand the physiopathology of infection such as the tissue tropism of the virus, its kinetics of dispersion and the presence or absence of associated pathological effects. In addition, the establishment of relevant preclinical models is essential to validate vaccine candidates and therapies identified in vitro and in mice. Human and Cynomolgus macaques (Macaca fascicularis) share immune and biological responses to infection by other arboviruses such as Chikungunya virus and Dengue virus.
We inoculated male and female cynomolgus macaques with different doses of ZIKA virus HPF-13 strain. Viremia were measured by RTqPCR over time on plasma and other body fluids such as saliva, urine and vaginal secretions. Clinical parameters were also monitored during the infection.
Characterization of NHP ZIKA infection model that we set up in Cynomolgus macaques will be presented and compared to other NHP models, which used other ZIKV strains or NHP species.

*Speaker

The organising EU funded Zika projects

ZIKAlliance is a multinational and multi-disciplinary research consortium coordinated by Inserm, the French National Institute of Health and Medical Research, and funded by the European Union's Horizon 2020 Research and Innovation Programme. The project started in October 2016 to conduct a 36-month cutting-edge research project during the ongoing outbreak of Zika virus infection (ZIKV) in Latin America and the Caribbean.

The consortium is coordinated by leading virologist Prof. Xavier de Lamballerie (Inserm, IRD, Aix-Marseille University), and it includes 53 partners, located in 18 countries.

In a global effort to combat what is a global threat that has affected 73 countries and territories worldwide (WHO Zika Situation Report, 13 October 2016), the three-year multidisciplinary ZIKAlliance project links large observational multicentre cohort studies with basic scientific research to focus on the following four key objectives:

- Impact of Zika virus infection during pregnancy and short and medium term effects on newborns;
- Natural history of Zika virus infection in humans and their environment in the context of other circulating arboviruses;
- Improving scientific knowledge about the virus, the mechanisms of infection and the immune response; evaluating diagnostic methods; identifying small molecules with antiviral potential;
- Building the overall capacity for preparedness research for future epidemic threats in Latin America and the Caribbean.

https://zikalliance.tghn.org/

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 734548.

ZIKAction – finding answers, preparing for the future

The international ZIKAction consortium has been established in South and Central America, the Caribbean and Europe with the complementary goals of:

- developing a multidisciplinary multinational **ready-to-act network capable of rapidly addressing any maternal and paediatric health research need arising from the ongoing Zika virus (ZIKV) outbreak** and

- conducting an interdisciplinary programme of research studies within this network to address key knowledge gaps relating to ZIKV epidemiology, natural history and pathogenesis, with a particular emphasis on maternal and child health.

ZIKAction proposes an integrated programme of epidemiological, clinical and pathobiological research studies to examine the strength and nature of the association between maternal ZIKV infection in pregnancy and adverse maternal and fetal outcomes, to elucidate the timing and mechanisms of vertical transmission of ZIKV and to investigate burden and natural history of congenital and acquired paediatric ZIKV infection.

Novel diagnostic methodologies are developed and validated within the network, including clinical algorithms optimised for rapid diagnosis of ZIKV in pregnant women, infants and children, supported by cohort study data.

ZIKAction creates a flexible infrastructure facilitating close linkages between the research programme, international and national public health organizations and other key stakeholders in the response to the ZIKV epidemic, with the capacity to embed new studies. ZIKAction has leveraged this flexibility in creating shared work packages for close collaboration with other EU-funded ZIKV projects including shared governance structures and ethics and regulatory oversight, integrated communications, harmonized clinical protocols and case definitions, internal data sharing mechanisms and a roadmap towards expanding data sharing, as well as a shared Latin American emerging infectious disease preparedness and response network.
http://zikaction.org/

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 734857.

ZikaPLAN (Zika Preparedness Latin American Network) brings together 25 leading research and public health organizations in Latin America, North America, Africa, Asia, and Europe, taking a comprehensive approach to tackle the Zika threat.
From urgent response to long-term capacity

- Addressing the urgent research knowledge gaps and needs in the current Zika outbreak to better understand the disease, prevent its spread and engage with the affected populations

- Building a sustainable preparedness and response capacity in Latin America for Zika and other emerging infectious diseases

The rapid impact of the Zika outbreak in Latin America requires a rapid worldwide response. The severity of the outbreak and the mutation of the virus have led to a great number of, as yet, unanswered research questions.

What is needed, in the short term, is the implementation of effective measures to answer these questions. To achieve this, health authorities need to learn more about the severity of the disease, its impact on public health, how effectively to prevent and stop its spread, and the best ways to manage and treat those who have been infected.

It has become clear, in this unprecedented Zika outbreak, that building local capacities is vital. In some of the regions where the virus struck, understanding the threat of this disease and the subsequent necessary and rapid implementation of measures to combat it, proved impossible, as the research infrastructure simply did not exist.

ZikaPLAN aims to address the Zika virus outbreak and the many research and public health challenges it poses. The approach it takes is comprehensive, encompassing epidemiological surveillance, clinical studies, the development of innovative diagnostic tools and control strategies, in addition to education and knowledge sharing.

https://zikaplan.tghn.org/

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 734584.

ZIKAVAX

Fast track development of a Zika vaccine based on measles vector.

ZIKAVAX is a collaborative project funded under the European Union's Horizon 2020 Research and Innovation Programme (H2020). The four-year project was initiated in October 2016 and has an overall budget of approximately € 5 million. The project is the joint effort of leading European experts from academia and industry with unique and specific technological expertise in viral vectors and vaccine development. ZIKAVAX is coordinated by the European Vaccine Initiative (EVI) and includes Institut Pasteur Paris, Themis Bioscience GmbH and the Commissariat à l'énergie atomique et aux énergies alternatives.

The ZIKAVAX project aims at developing a safe, effective, and affordable preventive vaccine against Zika virus infection. To achieve this goal, ZIKAVAX uses a delivery platform technology based on a measles vector (MV) with demonstrated proof of principle in humans and a preclinical track record of rapid adaptability and effectiveness for a variety of pathogens. In ZIKAVAX, following antigen selection and expression, immunisation studies were conducted with the Zika vaccine candidate in mice and will be conducted in a non-human primates challenge model that was developed by the consortium. The GMP production of a clinical lot for the selected MV-Zika vector will then be performed based on a previously established process using a scalable platform technology that results in high yield and purity using standard equipment, followed by toxicology studies in macaques in order to assess the reactogenicity and toxicity of the recombinant MV. Finally, the safety and immunogenicity of a recombinant MV-Zika vaccine candidate will be evaluated in healthy adults, including the identification of a suitable immunogenic dose of the recombinant vaccine and the demonstration of an acceptable reactogenicity profile. The vaccine candidate will have to induce in subjects with pre-existing immunity to measles vector significant levels of Zika virus-specific neutralising antibodies, and ideally also measurable CD4 and CD8-positive T cells.

The ultimate goal of ZIKAVAX is the demonstration of safety and immunogenicity of a recombinant measles-Zika vaccine candidate (MV-ZIKA) in adult volunteers in a phase Ia clinical trial.

"We are convinced that the use of the measles vaccine delivery platform, one of the safest and most efficacious vaccines available to date, will allow for a rapid and cost-effective development of a Zika vaccine" says Dr Frédéric Tangy, Head of the Viral Genomics and Vaccination Unit, Institut Pasteur.

www.zikavax.eu

EVI Institut Pasteur THEMIS DE LA RECHERCHE À L'INDUSTRIE cea

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 732432.

The stands

EVAg is a non-profit organisation dedicated to the **characterization / conservation / production / distribution / characterization** of biological materials in the field of virology.

For that purpose, EVAg maintains high control standards and develops appropriate methods directly relevant to human and animal virology. The EVAg consortium also ensures:

- that materials are supplied non-profitably or at cost to all bona fide applicants,
- that biosafety and security measures are appropriately addressed by the supplying and recipient labs,
- that the available materials meet the highest scientific standards in terms of quality and characterization,
- that the property of the provided materials remains with the originators.

EVAg is the first and currently, the only 21st century global virus collection conceived as a modern and innovative support organization for scientific research, education and disease control through human and veterinary health programmes.

The project objectives meet the needs of scientists, worldwide, by generating a carefully authenticated animal virus collection that is larger than any existing repository, and readily available to all laboratories that meet approved ethical, safety and security standards.

https://www.european-virus-archive.com/

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 653316.

Introducing REDe the research capacity and preparedness to tackle emerging infectious disease outbreaks in Latin America and Caribbean

Background: In 2013 the WHO stated that unless low-income countries become the generators, rather than the recipients, of health research data there will never be any real improvement in the devastating public health challenges these countries face. The Global Health Network was cited as an important agent for change in addressing this need.
The Global Health Network built an innovative digital platform, generating and supporting communities of practice focused on global health research. We have established a vast online knowledge-sharing resource, so far visited by more than 1.5 million frontline healthcare workers and researchers globally. Over 400,000 online modules were taken by users from our target countries using our Training Centre, which offers a wide range of high quality research skills courses. We also support skills development through regionally-led activities.

Here we present how our approach is applied to support preparedness for epidemic outbreaks in Latin America and the Carribean.

Methods: A community of practice (CoP) was set up for the 3 EU funded Zika Consortia on The Global Health Network platform (https://rede.tghn.org/). It provides a mechanism for research staff, across the consortia to work together, share ideas, methods and approaches to foster knowledge exchange and collaboration. The REDe CoP platform hosts training courses, help topics, templates, guidance – everything that is needed to run a good clinical study. The initial set of resources is available now, others are being developed as a result of Knowledge Gap Analysis. In addition, our online platform offers free participation in the Professional Development Scheme - a unique framework to track research skills development.

Results and Conclusions: The REDe capacity development CoP was launched in October 2016. We will present how this regionally-championed initiative is being taken up and what difference it is already making to the community of researchers.

Author: Nina Jamieson
Dept.: Centre for Tropical Medicine
Research Group: The Global Health Network
Research Group Director: Trudie Lang

https://rede.tghn.org/

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