Zika virus emergence in the Americas –
A potential threat to Europe?
WHO statement, 1 February 2016

“The Committee advised that the recent cluster of microcephaly cases and other neurological disorders reported in Brazil, following a similar cluster in French Polynesia in 2014, constitutes a Public Health Emergency of International Concern (PHEIC).”

I. Surveillance for Zika virus infection should be enhanced

II. The development of new diagnostics for Zika virus infection should be prioritized

III. Vector control measures and appropriate personal protective measures should be aggressively promoted and implemented
The number of suspected microcephaly cases in Brazil relied on a screening test that had very low specificity and therefore overestimated the actual number of cases by including mostly normal children with small heads.

<table>
<thead>
<tr>
<th>Cutoffs</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Estimated annual number of suspected cases (thousands)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil's Ministry of Health (up to Dec 8, 2015)</td>
<td>79.3%</td>
<td>92%</td>
<td>158</td>
</tr>
<tr>
<td>Brazil's Ministry of Health (after Dec 8, 2015)</td>
<td>93.8%</td>
<td>86%</td>
<td>46</td>
</tr>
<tr>
<td>Pan American Health Organization</td>
<td>96.1%</td>
<td>80%</td>
<td>29</td>
</tr>
<tr>
<td>Below -2 SD, InterGrowth standards</td>
<td>97.8%</td>
<td>85%</td>
<td>18</td>
</tr>
<tr>
<td>Below -3 SD, InterGrowth standards</td>
<td>99.9%</td>
<td>57%</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* Based on applying the InterGrowth standards to the distribution of livebirths by gestational age in Brazil. †Preliminary results based on a case series of 31 newborn babies with radiological evidence of brain abnormalities. ‡Calculated on the basis of sensitivity and the gestational age distribution of Brazilian newborn babies.

Victoria et al. (2016) Lancet
907 confirmed cases of microcephaly or other nervous system malformation among newborns have been reported in Brazil since 22 October 2015.
The increases of microcephaly cases in Pernambuco state, Brazil were registered 7-8 months after the first detection of Zika virus cases.

PAHO (March 2016)
Spatiotemporal spread pattern of Zika virus based on analysis of NS5, envelope, and complete genome sequences demonstrates recent introduction into Brazil.
So far, two genetic lineages are described: the African and the Asian lineage of Zika virus.
Congenital Zika virus infection and microcephaly

Mlakar et al. (2016) NEJM
Zika virus infection and fetal brain damage
The risk of microcephaly increases to about 1% when mothers are infected with Zika virus during the first trimester of pregnancy.
The incidence of Guillain-Barré syndrome cases during the French Polynesian outbreak was estimated to be 0.24 per 1000 Zika virus infections.
The geographic distribution of GBS cases in El Salvador indicates that there is a spatial and temporal association with Zika virus cases.
HPs, African lineage of Zika virus achieved peak plasma at least one order of magnitude lower those observed in animals infected with the Asian lineage of Zika virus.
Twenty-two pregnant NHPs with and without pre-existing anti-dengue immunity will be inoculated with low passage Asian and African lineage of Zika virus 15 days after insemination and monitored for birth defects.
Urgent questions / tasks

- Does the observed association of Zika virus infection with microcephaly and GBS simply reflect an increased incidence of infection or is it the result from a change in virulence or is it caused by co-factors?

- Will Zika virus become endemic and an enzootic transmission cycle develop in the Americas?
Course of Zika virus diagnostic parameters in flavivirus-naive individuals.

- ZIKV RNA in blood and urine
- Antibody titer (IgG and IgM) over time

Days after onset of symptoms:
- 0: Onset of symptoms
- 7, 14, 21, 28, 60, 120, 240, 480

BNITM (2016)
In NHPs, prolonged detection of viral RNA in urine and saliva after clearance from the blood was demonstrated.
There is growing evidence of sexual transmission of Zika virus.

Ortenzio et al. (2016) NEJM
Development and evaluation of specific and sensitive CE-IVD marked real-time PCR test kits for Zika virus detection:

The limit of detection for Zika virus using the RealStar® Zika Virus RT-PCR Kit 1.0 is 0.61 copies/μl (95% confidence interval 0.39 to 1.27 copies/μl).

Viruses tested to demonstrate the analytical specificity of the RealStar® Zika Virus RT-PCR Kit 1.0:

<table>
<thead>
<tr>
<th>Organisms</th>
<th>FAM Channel (Zika virus)</th>
<th>JOE Channel (Internal Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese encephalitis virus</td>
<td>Negative</td>
<td>Valid</td>
</tr>
<tr>
<td>St. Louis encephalitis virus</td>
<td>Negative</td>
<td>Valid</td>
</tr>
<tr>
<td>West Nile virus</td>
<td>Negative</td>
<td>Valid</td>
</tr>
<tr>
<td>Yellow fever virus</td>
<td>Negative</td>
<td>Valid</td>
</tr>
<tr>
<td>Murray Valley encephalitis virus</td>
<td>Negative</td>
<td>Valid</td>
</tr>
<tr>
<td>SEBOV Gulu</td>
<td>Negative</td>
<td>Valid</td>
</tr>
<tr>
<td>ZEBOV Mayinga</td>
<td>Negative</td>
<td>Valid</td>
</tr>
<tr>
<td>MARV Musoke</td>
<td>Negative</td>
<td>Valid</td>
</tr>
<tr>
<td>Dengue virus serotype 1</td>
<td>Negative</td>
<td>Valid</td>
</tr>
<tr>
<td>Dengue virus serotype 2</td>
<td>Negative</td>
<td>Valid</td>
</tr>
<tr>
<td>Dengue virus serotype 3</td>
<td>Negative</td>
<td>Valid</td>
</tr>
<tr>
<td>Dengue virus serotype 4</td>
<td>Negative</td>
<td>Valid</td>
</tr>
</tbody>
</table>

BNITM and altona Diagnostics (2016)
Most of the in-house assays showed comparably high analytical sensitivity with around 5 copies per reaction.
We projected the estimated risk of false-negative test results for Zika virus RNA to 20% upon usage of highly sensitive assays with a detection threshold of 5 copies per reaction.
Development and evaluation of specific and sensitive CE-IVD marked Zika virus ELISA:

**IgM**

**IgG**
Seroconversion panels confirmed the high sensitivity of the Zika virus ELISA:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gender</th>
<th>Age in years</th>
<th>Days after onset of symptoms</th>
<th>PCR Material</th>
<th>PCR Results</th>
<th>EUROMMUN ELISA IgG Results</th>
<th>EUROMMUN ELISA IgM Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>f</td>
<td>27</td>
<td>3 Serum</td>
<td>pos.</td>
<td>2,2</td>
<td>pos.</td>
<td>4,8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17</td>
<td></td>
<td>3,0</td>
<td>pos.</td>
<td>0,8</td>
</tr>
<tr>
<td>Patient 2</td>
<td>m</td>
<td>45</td>
<td>7 Urine</td>
<td>pos.</td>
<td>1,3</td>
<td>pos.</td>
<td>4,0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td></td>
<td>2,2</td>
<td>pos.</td>
<td>1,1</td>
</tr>
<tr>
<td>Patient 3</td>
<td>m</td>
<td>4</td>
<td>3 Urine</td>
<td>pos.</td>
<td>0,1</td>
<td>neg.</td>
<td>0,2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td>0,8</td>
<td>bl.</td>
<td>1,7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td></td>
<td>2,1</td>
<td>pos.</td>
<td>1,6</td>
</tr>
<tr>
<td>Patient 4</td>
<td>m</td>
<td>4</td>
<td>11 Urine</td>
<td>pos.</td>
<td>2,6</td>
<td>pos.</td>
<td>7,5</td>
</tr>
<tr>
<td>Patient 5</td>
<td>f</td>
<td>28</td>
<td>11 Urine</td>
<td>pos.</td>
<td>4,8</td>
<td>pos.</td>
<td>5,6</td>
</tr>
</tbody>
</table>

BNITM and EUROIMMUN (2016)
Zika virus diagnostic algorithm in Germany

1. Stay in an area epidemic for Zika virus during the last 3 weeks?
   - Yes
   - No
2. Sex partner with stay in an area epidemic for Zika virus during the last 3 weeks?
   - Yes
   - No
3. Actual or previous symptoms of a Zika virus infection?
   - Yes
   - No
4a. Known pregnancy?
   - Yes
   - No
4b. From day 28 after return:
   - Serology from serum
   - PCR from urine

Until day 7 after onset of symptoms:
- PCR from serum or plasma AND
- PCR from urine

8 to 28 days after onset of symptoms:
- Serology from serum (IgG/IgM) AND
- PCR from urine

Diagnostic for Zika virus infection not necessary
A virus diagnostic algorithm of the CDC

Antibody testing
(Serum collected 2 - 12 weeks post travel)
and laboratory findings of imported Zika virus infections diagnosed at the WHO CC until the beginning of the epidemics in the Americas.

<table>
<thead>
<tr>
<th>year</th>
<th>month</th>
<th>clinical findings</th>
<th>Zika virus diagnostics</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>November</td>
<td>52-year-old woman, fever, polyarthralgia, rash</td>
<td>IgG+, IgM+, VNT+, RT-PCR-</td>
<td>Tappe et al. (2013) Eurosurveillance</td>
</tr>
<tr>
<td></td>
<td>December</td>
<td>31-year-old woman, fever, polyarthralgia, myalgia, rash, Lymphopena, neutropenia</td>
<td>IgG+, IgM+, RT-PCR+</td>
<td>Wæhre et al. (2014) Emerg Infect Dis</td>
</tr>
</tbody>
</table>
Functional T cell activation was seen during the acute phase of virus disease (ZVD) characterized by respective cytokine levels, followed by a decrease in the reconvalescent phase of ZVD.
Recovery from ZIKV infection is associated with restoration of normal numbers of immune cells in the periphery as well as with normal function of antigen-presenting cells.
Urgent questions / tasks

Simple point-of-care diagnostics (Ag, Ab or RNA) will be needed.

Harmonization of VNTs (reference ZIKV strains?, PRNT50 or PRNT90, FRNTs, endpoint titration?)
*Aedes aegypti* populations with widespread resistance to insecticides
More than 19 mosquito species were found to be infected with Zika virus

* Aedes metallicus (Africa)
  * Aedes opok (Africa)
  * Aedes taylori (Africa)
  * Aedes unilineatus (Africa)
  * Mansonia uniformis (Africa)

* Aedes aegypti aegypti (global)
  * Aedes albopictus (global?)
  * Aedes polynesiensis (Polynesia)
  * Aedes hensilii (Polynesia)
Important intraspecies differences for the vector competence of *Aedes aegypti* and *Aedes albopictus* after 14 days of oral exposure to ZIKV.
European *Aedes aegypti* and *Aedes albopictus* populations are competent vectors for Zika virus.
Aedes albopictus is an invasive mosquito species in Europe:

ECDC (2010 and 2016)
Modeling of suitable areas for Zika virus in Europe

- Distribution of *Aedes albopictus*
  - Probability of occurrence
- Conservative ZIKV model (28°C)
  - Days accumulating 14 days ≥ 28°C
- Generous ZIKV model (25°C)
  - Days accumulating 14 days ≥ 25°C

Luehken, BNITM (2016)
The conservative model (28°C), demonstrates that coastal areas of France, Italy, Greece, and Turkey are suitable for Zika virus.
The generous model (25°C), demonstrates that most parts of the Mediterranean countries are suitable for Zika virus.

Luehken, BNITM (2016)
Indigenous and invasive mosquito species from Germany will be infected with low passage Asian and African lineage of Zika virus and tested for virus replication and transmission.

- **April - May**: Infection of *Aedes aegypti* (lab strain) and *Culex pipiens* (lab strain)
- **June - August**: Collection of *Culex torrentium*, *Aedes vexans*, *Aedes albopictus*, and *Aedes japonicus* eggs
- **September - December 2017**: RNA isolation of all samples & qPCR analysis TCID50

Heitmann and Jansen (2016) BNITM
Returning viremic travellers may ignite autochthonous infections in European countries. Therefore, it will be important to enhance vigilance towards the early detection of imported cases of ZIKV infection in Europe, in order to reduce the risk of autochthonous transmission.

„Mit der Meldepflicht für Arboviren sorgen wir außerdem dafür, dass etwa eine Zika-Infektion bei Reiserückkehrern in Deutschland besser überwacht werden kann. Damit gewinnen die Gesundheitsämter vor Ort wertvolle Zeit zum schnellen Handeln."
Urgent questions / tasks

Targeted and sustainable vector control measures

Is Zika virus able to infect and be transmitted by other mosquito genera (e.g. *Culex*, *Mansonia*, *Anopheles*)?