Emerging and endemic arboviruses: Challenges for laboratory surveillance

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Emerging diseases

*Microbial Threats to Health in the United States*

1992, Instituto de Medicina (USA)
(Joshua Lederberg & Robert Shope)

- **Emerging diseases:**
  new diseases / new pathogens (or new strains) / expansion to new territories

- **Re-emerging diseases:**
  diseases previously “under control” but re-appear increasing the incidence
Emerging & re-emerging diseases, 1996-2017

Emerging & re-emerging diseases, 1996-2017
Emerging Arboviruses

Epidemiological Alert
Zika virus infection
7 May 2015

The Pan American Health Organization (PAHO) / World Health Organization (WHO) recommends its Member States establish and maintain the capacity for Zika virus infection detection, clinical management and an effective public communication strategy to reduce the presence of the mosquito that transmits this disease, particularly in areas where the vector is present.

Epidemiological Alert
Neurological syndrome, congenital malformations, and Zika virus infection. Implications for public health in the Americas
1 December 2015

Given the increase of congenital anomalies, Guillain-Barré syndrome, and other neurological and autoimmune syndromes in areas where Zika virus is circulating and their possible relation to the virus, the Pan American Health Organization / World Health Organization (PAHO/WHO) recommends its Member States establish and maintain the capacity to detect and confirm Zika virus cases, prepare healthcare facilities for the possible increase in demand at all healthcare levels and specialized care for neurological syndromes, and to strengthen antenatal care. In addition, Member States should continue efforts to reduce the presence of mosquito vectors through an effective vector control strategy and public communication.

Epidemiological Alert
Increase of microcephaly in the northeast of Brazil
17 November 2015

Given the unusual increase in cases of microcephaly in some northeast states of Brazil, the Pan American Health Organization (PAHO) / World Health Organization (WHO) calls upon Member States to remain alert to the occurrence of similar events in their territories and to notify its occurrence through the channels established under the International Health Regulations (IHR).

Media centre

WHO statement on the first meeting of the International Health Regulations (2005) (IHR 2005) Emergency Committee on Zika virus and observed increase in neurological disorders and neonatal malformations
1 February 2016

Based on this advice the Director-General declared a Public Health Emergency of International Concern (PHEIC) on 1 February 2016. The Director-General endorsed the Committee’s advice and issued them as Temporary Recommendations under IHR (2005). The Director-General thanked the Committee Members and Advisors for their advice.
(Re) Emerging

DENV (endemic)

YFV (endemic, re-emerging)
South-east Brazil:

**December 2016 – June 2017**
- 792 confirmed cases
- 274 deaths [CFR: 34.6%]
- 4 states: SP, MG, RJ, ES

**July 2017 - June 2018***
- 1266 confirmed cases
- 415 deaths [CFR: 32.8%]
- 4 states: SP, MG, RJ, ES

2015: 27 cases in Peru, Brazil
2016: 113 cases in Peru, Brazil, Colombia,
2017: 819 cases in Brazil, Peru, Bolivia, Ecuador, Colombia, Suriname, Fr. Guiana

Distribution of confirmed yellow fever cases by epidemiological week (EW). Brazil, 2016 – 2018*
Emerging

CHIKV (Emerging, 2013)

ZIKV (Emerging, 2015)
### Other arboviruses affecting humans

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<th>Virus</th>
<th>Family</th>
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<th>Vertebrate hosts</th>
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<tr>
<td>Chikungunya</td>
<td>Togaviridae</td>
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<td>Primates, birds, cattle, and rodents</td>
<td>Africa, Asia, Europe, Americas, Oceania</td>
</tr>
<tr>
<td>Mayaro</td>
<td>Togaviridae</td>
<td>Mosquitoes: Haemagogus spp.</td>
<td>Primates, other mammals, birds</td>
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<tr>
<td>Ross River</td>
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<tr>
<td>O’nyong-nyong</td>
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<td>?</td>
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<tr>
<td>Sindbis</td>
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<td>Mosquitoes: Aedes, Culex, and Culiseta spp.</td>
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<tr>
<td>Western equine</td>
<td>Togaviridae</td>
<td>Mosquitoes: Culex, Aedes, Ochlerotatus, and Culex spp.</td>
<td>Birds, horses, other mammals</td>
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<td>encephalitis</td>
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<tr>
<td>Venezuelan equine</td>
<td>Togaviridae</td>
<td>Mosquitoes: Culex, Ochlerotatus, Anopheles, Mansonia, Psorophora, Aedes spp. and others</td>
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<td>encephalitis</td>
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<td>Dengue</td>
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<td>Mosquitoes: Aedes spp</td>
<td>Primates</td>
<td>Asia, Americas, Africa, Europe, Oceania</td>
</tr>
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<tr>
<td>Yellow Fever</td>
<td>Flaviviridae</td>
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<td>Europe, Oceania, South America, Africa</td>
</tr>
<tr>
<td>West Nile</td>
<td>Flaviviridae</td>
<td>Mosquitoes: Culex spp, Birds, Pigs</td>
<td>Africa, Asia, Europe, Oceania, Americas, Asia, Oceania</td>
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<td>Zika virus</td>
<td>Flaviviridae</td>
<td>Mosquitoes: Aedes spp</td>
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<tr>
<td>Rocio</td>
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<td>Mosquitoes: Psorophora and Aedes spp</td>
<td>South America</td>
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<td>St. Louis encephalitis</td>
<td>Flaviviridae</td>
<td>Mosquitoes: Culex spp, Ticks: Hemaphysalis spp</td>
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<tr>
<td>Kyasanur Forest disease</td>
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<td>Ticks: Dermacentor and Ixodes spp Mosquitoes?</td>
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<td>Omsk hemorrhagic fever</td>
<td>Flaviviridae</td>
<td>Ticks: Ixodes spp</td>
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<td>Tick-borne encephalitis</td>
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<td>Ticks: Ixodes spp</td>
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<td>Sandfly fever</td>
<td>Bunyaviridae</td>
<td>Sandflies: Phlebotomus spp</td>
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<td>Rift Valley fever</td>
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<td>Mosquitoes: Aedes, Ochlerotatus, Strengomyia, Anopheles, Culex, Neomelaniconion, Eretmapodites and others</td>
<td>Africa, Asia</td>
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<td>La Crosse encephalitis</td>
<td>Bunyaviridae</td>
<td>Primates, Rodents, Other Mammals</td>
<td>Americas, Asia</td>
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<tr>
<td>Crimean-Congo hemorrhagic fever</td>
<td>Bunyaviridae</td>
<td>Mosquitoes: Aedes spp, Ticks: Hyalomma spp</td>
<td>Europe, Asia, Africa</td>
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<tr>
<td>Oropouche</td>
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<td>Midges: Culicoides sp</td>
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<td>Severe febrile thrombocytopenia syndrome</td>
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<td>Bluetongue</td>
<td>Reoviridae</td>
<td>Mosquitoes: Culicoides spp</td>
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</table>
Mayaro and other arboviruses...

**Mavaro virus**
- MAYV reported (virus isolates, human cases)
- Reported MAYV cases
- Case from this report
- Serologic evidence of MAYV circulation

Emerg Infect Dis, 2012, 18:695-6

**Oropouche virus**


**EEE virus**


**VEE virus**
Surveillance and early response: Role of the laboratory
Surveillance and early response

International Health Regulations:

- Recommends countries to maintain active surveillance of diseases and public health events.
- Urges to strengthen and respond quickly to events of international dispersion and contain any threat to public health.

Pathogens under elimination or control: viruela, poliovirus, etc...

Emerging or re-emerging pathogens (Zika, YFV, SARS, MERS, Flu...)

Early Warning System
Surveillance and early response

International Health Regulations:

- Core capacity # 8 of the IHR (2005) obligates WHO Member States to establish mechanisms to provide reliable and timely identification and characterization of infectious agents and other hazards that may cause public health emergencies of national and international interest, including sending specimens to the appropriate laboratories if necessary.

Pathogens under elimination or control: viruela, poliovirus, etc...

Emerging or re-emerging pathogens (Zika, YFV, SARS, MERS, Flu...)

Early Warning System
IHR, Core capacity # 8: Critical laboratory elements

1. Capacity to diagnose priority pathogens
2. Quality management
3. Management of biological risk
4. Collection and transport of samples
5. Laboratory-based surveillance
6. Laboratory networks
IHR, Core capacity # 8: Critical laboratory elements

1. Capacity to diagnose priority pathogens

2. Quality management

3. Management of biological risk

4. Collection and transport of samples

5. Laboratory-based surveillance

6. Laboratory networks
Standardization of processes

Processing
- Type of sample
- Sample conservation
- Sample transportation
- Type of test
- Equipment and Reagents
- Reference materials

Algorithms
- Sequential Vs simultaneous
- Singleplex Vs Multiplex
- Differentials

Implementation
- Training
- SoPs
- Equipment calibration
- Interpretation of results
- Limitations
- QC
- EQAP
- Biosecurity
- Biosafety
- Maintenance
- Waste disposal
Standardization of processes

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**Algorithms**
- Sequential Vs simultaneous
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- Differentials

**Interpretation**
Laboratory algorithms

Infection dynamics: Replication / Immune response

Slide adapted from Dr. Jorge Muñoz, CDC
Pto. Rico
Processing algorithms

• The laboratory algorithms are **NOT** static and should be adjusted depending on the needs, epidemiological profile and to respond to emergencies
Dengue

Caso sospechoso de dengue

Muestra de suero tomada 1-5 día de síntomas

ELISA NS1

+ Confirma

- Descarta

Tipificación por RT-PCR

- Positivo

- Negativo

Aislamiento Viral*

- Positivo

Muestra de suero tomada ≥ 6 día de síntomas

ELISA IgM

+ Confirma

- Descarta

Adaptado de: Laboratorio de Arbovirus-Grupo Virología. INS Colombia
**Muestra de caso sospechoso**

**CHIKV**

Inicio de síntomas vs. toma de muestra

- ≤8 días
  - PCR+IgM
  - RT-PCR DENV
    - Pos
    - Neg
    - rRT-PCR CHIKV
      - Neg
      - Pos

- >8 días
  - IgM DENV
    - Neg
    - Pos

- Envío de 25 muestras positivas al centro colaborador: Aislamiento viral (BSL3), secuenciación, PRNT
- Envío del 10% de muestras negativas

- 2ºa muestra
  10-14 días después de la muestra aguda

- Pos
- Neg
Algorithm for integrated surveillance

Algorithm A: Sequential

Serum sample collected 1-5 day of symptoms onset

RT-PCR ZIKV

Positive

Negative

RT-PCR DENV

Positive

Negative

RT-PCR CHIKV

Positive

Negative
Algorithm for integrated surveillance

**Algorithm B: Parallel**

Serum sample collected 1-5 day of symptoms onset

- **ZIKV**
  - RT-PCR ZIKV
    - Positive
    - Negative

- **DENV**
  - RT-PCR DENV
    - Negative
    - Positive

- **CHIKV**
  - RT-PCR CHIKV
    - Negative
    - Positive
Algorithm for *integrated* (serological) surveillance

- Serum sample taken $\geq 6$ days after onset of symptoms
- IgM (ELISA)

**Algorithm C:**

- **DENV Positive**
  - **ZIKV Negative**
    - Presumptive DENV (recent)

- **DENV Positive**
  - **ZIKV Positive**
    - Flavivirus infection (recent)

- **DENV Negative**
  - **ZIKV Positive**
    - Presumptive ZIKV (recent)
Processing algorithms

- The laboratory algorithms are **NOT** static and should be adjusted depending on the needs, epidemiological profile and to respond to emergencies.

  Take into account new findings, biological evidence and performance of new assays.
Laboratory algorithms

Infection dynamics: Replication / Immune response

Slide adapted from Dr. Jorge Muñoz, CDC Pto. Rico
Algorithm for laboratory confirmation of yellow fever (YF) cases

Suspected YF case

- Symptom onset vs. Sample collection

  - ≤10 days from symptom onset

    - YF RT-PCR
      - Positive: Confirmed YF case
      - Negative: YF IgM ELISA
    - Negative: IgM differential diagnosis
      - Positive: Recent flavivirus infection
      - Negative: Probable YF case

    - YF IgM ELISA
      - Positive: sample collected ≤7 days from symptom onset
        - Positive: Inconclusive
        - Negative: Exclude YF
      - Negative: sample collected ≥8 days from symptom onset
        - Positive: Recent flavivirus infection
        - Negative: Probable YF case

1. No YF vaccination within 30 days or unknown YF vaccination history.
2. Laboratories that only have the capacity to perform RT-PCR or IgM ELISA should test samples with the available technique. Results should be interpreted according to the algorithm.
3. RT-PCR sensitivity is higher in the first 10 days from symptom onset. However, detection beyond 10 days has been reported, in particular in severe (and fatal) cases.
4. Must include dengue virus as well as other flaviviruses depending on the epidemiological situation of the area/country.
5. Consider performing PRNT in a reference laboratory. This result does not rule out yellow fever. Thus, in areas where no YF circulation has been described recently, this should prompt an investigation.
6. A positive IgM test in a single sample is not confirmatory. Additional clinical and epidemiological criteria must be used for the final interpretation of the case, in particular in areas where no YF circulation has been described recently.
7. A second sample should be requested and tested according to the algorithm.
8. Cases should be investigated and clinical differential diagnosis performed.
Processing algorithms

- The laboratory algorithms are **NOT** static and should be adjusted depending on the needs, epidemiological profile and to respond to emergencies

  For early detection of emerging agents, the **negative** samples are as important as the positive ones
Algorithms to detect emerging arboviruses...

Serum sample collected 1-5 day of symptoms onset

RT-PCR ZIKV

Positive

Negative

RT-PCR DENV

Positive

Negative

RT-PCR CHIKV

Positive

Negative

How far to go?

?????
Algorithms to detect emerging arboviruses...

• Additional assays…(*singleplex?)

• Generic assays
  o Panflavivirus
  o Panalphavirus
  o Panbunyavirus
Muestra de suero tomado 1-7 día de síntomas

**Panflavi**
- **Positivo**: DENV, ZIKA
- **Negativo**

**Panalpha**
- **Positivo**: CHIKV, MAYV
- **Negativo**

**Panbunya**
- **Positivo**: OROV
- **Negativo**

“Generic” algorithms (1)
Muestra de suero tomada 1-7 día de síntomas

- **DENV**
  - RT-PCR DENV
    - Positivo
    - Negativo

- **CHIKV**
  - RT-PCR CHIKV
    - Negativo
    - Positivo

- **ZIKV**
  - RT-PCR ZIKV
    - Negativo
    - Positivo

**PAN Flavi/Alpha/Bunya**
Muestra de suero tomada 1-7 día de síntomas

- DENV
  - RT-PCR DENV
    - Positivo
    - Negativo

- CHIKV
  - RT-PCR CHIKV
    - Negativo
    - Positivo

- ZIKV
  - RT-PCR ZIKV
    - Negativo
    - Positivo

Ailsamiento viral

"Generic" algorithms (3)
Processing algorithms

- The laboratory algorithms are **NOT** static and should be adjusted depending on the needs, epidemiological profile and to respond to emergencies.

  In case of emergencies due to natural disasters, the differential diagnosis must be considered with other types of agents.
Algorithm for the differential diagnosis of arbovirus and leptospira infection in areas of documented co-circulation and post-emergency areas at risk

**Algorithm 1: suspected case (viremic/bacteremic phase)**

Serum sample taken ≤ 5 days after onset of symptoms

- Dengue NS1 ELISA
  - Positive
  - Negative

Typing by RT-PCR

- Positive
  - DENV infection

- Negative
  - Does not rule out

Arbovirus RT-PCR (serum sample)

- Negative
- Positive

PCR Leptospira

Whole blood sample taken in EDTA ≤ 10 days after onset of symptoms

Differential diagnosis:
- Bartonella
- Rickettsia
- Ehrlichia

(1) Perform a leptosiroa IgM ELISA with this serum sample and a second sample collected at least 14 days after the first to test for seroconversion
(2) A minimum sample volume of 5ml should be collected to obtain at least 1ml of serum for arboviruses testing and 1ml of serum for leptospira testing
(3) Sensitivity can vary depending on the serotype
(4) Molecular detection can be performed sequentially (singleplex, starting with the most probable agent according to clinical criteria) or in parallel (multiplex)
(5) ZIKV can also be detected by RT-PCR in urine from day 1 to day 15 (on average)
Processing algorithms

• The laboratory algorithms are **NOT** static and should be adjusted depending on the needs, epidemiological profile and to respond to emergencies

For the differential diagnosis of other pathologies considered in the IHR and that are in the process of elimination: Measles vs Zika
Paciente con fiebre y rash detectado por el sistema de vigilancia S/R (según definición de caso)

Colectar muestra de orina y suero

Procesar S/R

Continuar estudio S/R

Algoritmo detección ZIKV

Muestra de suero ≤5 días
Algoritmo A or B

Muestra de suero ≥ 6 días
Algorithm C
Comentarios finales

• Adequate surveillance allows monitoring endemic pathogens
• Efficient mechanisms are required to identify new events, new agents (viruses), or new variants with pandemic potential.

SURVEILLANCE OF UNUSUAL CASES
The laboratory is critical to confirm (or rule out) new agents: Zika, Mayaro, Oropouche, EEV, West Nile ...

LSPs must be prepared to detect and report new agents in a timely manner (mandatory notification within 24 hours, RSI)

However, the detection capacity does not refer only to the installed capacity; It implies the possibility of having access to a laboratory that has the capacity (Networking!)
Comentarios finales

• A good laboratory diagnosis depends on a good sample and a well recognized case ...

• Surveillance results should not be used / expected to make medical decisions! (the clinical diagnosis should be prioritized!)

• Articulation of the laboratory with the epidemiology and clinical components is essential to ensure an appropriate response to the IHR
Thank you!

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