Detection and identification of travel-acquired Zika virus infections

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• Background
• Case introductions
• Results
• Conclusions
- Fever
  - 1-3 days
- Maculopapular rash
  - 3-7 days
- Malaise/fatigue
  - 5-10 days
- Body aches or joint pain/swelling
  - 3-10 days*

Maculopapular rash, Cherabuddi et al., 2016, under review
Samples

- January – March 2016

- Recent travel history
  - Haiti (n=2)
  - Colombia (n=1)

- Whole blood/serum
- Saliva swab
- Urine
Testing

- Sample preparation
  - Whole Blood (Serum – 1 case)
  - Saliva
  - Urine

1. Culture methods
2. Molecular methods

Culture
MRC-5, LLC-MK2, Vero E6, and C6/36* cell lines

RT-PCR

RNA Extract

RT-PCR and real-time RT-PCR
Zika virus detection

- **Cell culture**
  - Observe changes in cells induced by virus infection
  - Cytopathic effects (CPE)

- **Molecular detection**
  - Not necessarily detecting infectious virus
  - +ssRNA
Culture methods

• Why culture?
  • Low virus concentration

• Cell lines used: MRC-5
  LLC-MK2
  Vero E6
  C6/36 – only for Albert’s samples

• 35-37°C
  • C6/36 at 28°C
• Grown up to 28 days
Cell culture

Vero E6

LLC-MK2

C6/36

MRC-5
Molecular detection

- Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)
  - Exponential amplification of a highly specific/conserved sequence
  - Primers and reagents
  - Binary results – is the sequence present or not
Molecular detection

- Real-time RT-PCR
  - Detection of amplification after each cycle via fluorescent probe
  - Qualitative
  - Lower Ct value = more +ssRNA present in sample
    - Threshold Cycle (Ct) value
Cases

- Case 1: Alberta missioned in Haiti
- Case 2: Albert visited Colombia
- Case 3: Dave traveled to Haiti
Case 1: Alberta

- Worked in Haiti for 2 weeks in January

- Symptom onset 2 days after returning to UF

- Common symptoms
  - Rash – ankles, spread upward

- Provided blood sample, saliva swab, and urine sample when rash appeared
Alberta’s test results

- RT-PCR: Blood, saliva and urine Zika positive

- Cultured in cells
  - Recovered infective zika virus from saliva and urine
  - 9 days post-infection (dpi) to 16dpi
  - LLC-MK2 and MRC-5 cell lines

- Real-time RT-PCR:
  - Saliva and urine MRC-5, 16dpi Ct= 4 and 13
  - Urine LLC-MK2, 16dpi Ct= 8
Alberta’s samples in culture

Mock infected, LLC-MK2 9 dpi

Saliva, LLC-MK2 9 dpi

Urine, LLC-MK2 9dpi
Alberta’s follow-up

- Saliva swab and urine sample 5 days after initial sample
  - Urine still positive (RT-PCR)
  - Saliva and urine grown in culture RT-PCR positive

- Saliva swab and urine sample 14 days after initial sample
  - RT-PCR negative
  - Grown in culture, urine RT-PCR positive, 20dpi
Case 2: Albert

- Colombia for 5 days, March
- Symptom onset 7 days after returning to the swamp
- Symptoms
  - Fever
  - Rash – torso
  - Severe, persistent arthralgia
- Provided blood, saliva swab, and urine sample when rash appeared
Albert’s results

- RT-PCR: Blood, saliva, and urine Zika positive
  - Cultured in cells
    - Recovered infective zika virus from blood, saliva and urine
    - 10 dpi
    - Blood and saliva, C6/36 and LLC-MK2
    - Urine, LLC-MK2 and Vero E6
  - Real-time RT-PCR
    - Blood and saliva, C6/36, 10dpi Ct= 15 and 23
    - Blood, saliva, and urine, LLC-MK2, 10dpi Ct= 20, 13, and 24
    - Urine, Vero E6, 10dpi Ct= 18
Albert’s sample in culture

Mock-infected LLC-MK2 cells  LLC-MK2 cells: mixed infection

Cherabuddi et al., 2016; under review
Albert’s co-infection

- Plaque assay
- Urine LLC-MK2, 10dpi
- Real-time RT-PCR
  - Zika
    - Ct = 18
  - Chikungunya
    - Ct = 33

White SK, 2016; unpublished
Case 3: Dave

- Haiti for 11 days, January
- Symptoms started the evening he returned to UF

- Common symptoms
  - Rash – torso, spread
  - Conjunctivitis

- Provided serum, saliva swab, and urine sample when rash appeared
  - Serum and urine sent to State Diagnostic Lab

Dave got Dengue
Don’t be like Dave
Dave’s results

- RT-PCR
  - State Lab - Blood was Dengue 2 positive, Zika negative
    - Urine Dengue 2 and Zika negative
  - Lednicky Lab - Blood was Dengue 2 positive, Zika negative
    - Saliva and urine Dengue 2 negative, Zika positive

- Culture
  - Recovered infective zika virus from saliva and urine
  - Both in MRC-5
  - Urine only in LLC-MK2
  - 16-19dpi
Dave’s results continued

- Zika virus Real-time RT-PCR
  - Saliva MRC-5 19dpi Ct= 18
  - Urine MRC-5 19dpi Ct= 20
  - Urine LLC-MK2 16dpi Ct= 12
Dave’s sample in culture
Dave’s follow-up

- Saliva swab and urine sample 36 days after symptom onset
  - RT-PCR negative
  - Culture negative
  - Real-time RT-PCR negative
Conclusions

• Imported Zika virus cases
  • Active transmission in Haiti and Colombia

• Zika virus presence in samples
  • Persistence still being investigated
  • Whole blood vs. plasma
  • Urine testing less invasive

• No commercial diagnostic
  • State/CDC testing overwhelmed
Co-infections questions

- Zika co-infections
  - Stop at identification of one pathogen
    - Differential diagnosis challenging
  - Antibody-dependent enhancement
- Chronology of infections, sequential or simultaneous
- One or multiple mosquitoes
References


