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Zika virus detected in the human eyes during outbreak investigation in Thailand, September 2016; a potential of person-to-person transmission of Zika virus

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Background: Zika virus (ZIKV), first isolated in 1947, is currently spreading rapidly through Latin America, the Caribbean and Asia. Multiple routes of ZIKV transmission are documented. The transmission is mainly vector-borne by *Aedes* mosquito and other is a person-to-person transmission. Few routes of person-to-person transmission are well demonstrated including mother-to-fetus/newborn, sexually contact and blood transfusion. In September 2016, the scientists described the ZIKV in the mice's tear and eyes. In additional, there was a report of suspicious eye contact transmission between human in USA. Thus eye contact is a questionable for possible transmission. In September 2016, we conducted laboratory investigation of eyes specimens with aim to demonstrate the ZIKV detectable in the human eyes.

Material/methods: A suspected ZIKV infection cases were required to collect blood, urine and saliva for ZIKV RT-PCR. Once the conjunctivitis was presenting, we additionally collected eye-swab in both left and right eyes. A polyester-tip-applicator was used for subconjunctival swab and kept in 1 mL of universal viral transport medium with cold chain. Blood, urine, saliva and eye-swab samples from the same patients were submitted to specific ZIKV-RNA detection. All specimens were initially screened by real-time RT-PCR for ZIKV using commercial kit and CDC protocol, with samples positive in these assays will be further confirmed by nested RT-PCR and sequencing on NS5 gene. Whole genome sequencing was also done by Next Generation machine.

Result: Totally 7 cases were identified from 2 provinces in north eastern and southern during outbreak investigation in September 2016. All cases were presenting with rash and conjunctivitis. Six cases were female and age range from 10 – 56 years old. The onset and time of specimen collection was 2-5 days. All 7 cases were ZIKV detected by real-time PCR of both commercial kit and US CDC protocol. Urine was 100% detectable, followed by saliva (6/7) and plasma/whole blood (4/7). Three cases were ZIKV detectable in eye swabs which collected swab in 2, 2 and 5 days after onset. Of these, 2 cases detected from commercial kit (Ct 33.46-34.13), 3 cases detected from US CDC protocol (ct 32.91–36.68) and 3 cases detected from nested protocol. The degree of erythematous conjunctivitis was associated with positive eye-swab. Furthermore 3 cases were positive ZIKV in all 4 different type of specimens (blood/urine/saliva/eye-swab). ZIKV sequences of different types of specimens from the same patient showed 100% identity of NS-5 gene (241 nucleotides). One urine sample (Ct 23.87 by commercial kit) was successfully undergone whole genome sequencing by Next Generation machine.

Conclusion: Here, we described the presence of infectious ZIKV from eye swabs, plasma, saliva and urine of acute onset in Thailand. From this finding, eye secretion in conjunctivitis of ZIKV infected case is potentially transmitted by person-to-person.

Sequence of 241nucleotides from 3 different specimens; saliva/eye-swab/urine compare to WGS urine in same person

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CLUSTAL W (1.99) multiple sequence alignment

Zika/HU/SI163858SV/Saliva/MDH/2016     AGAGCTGTGTGTACAACATGATGGGAAAAAGAGAAAAGAAACAAGGGGAATTTGGAAAGG
Zika/HU/SI163858ESR/Eye-Swab/MDH/2016  agagctgtgtgtacaacatgatgggaaaaagagaaaagaaacaaggggaatttggaaagg
Zika/HU/SI163851U/Urine/MDH/2016       agagctgtgtgtacaacatgatgggaaaaagagaaaagaaacaaggggaatttggaaagg
Zika/HU/SI163851/Urine/MDH/NGS/2016    agagctgtgtgtacaacatgatgggaaaaagagaaaagaaacaaggggaatttggaaagg
                                       ************************************************************

Zika/HU/SI163858SV/Saliva/MDH/2016     CCAAGGGCAGCCGCGCCATCTGGTATATGTGGCTAGGGGCTAGATTTCTAGAGTTCGAAG
Zika/HU/SI163858ESR/Eye-Swab/MDH/2016  ccaagggcagccgcgccatctggtatatgtggctaggggctagatttctagagttcgaag
Zika/HU/SI163851U/Urine/MDH/2016       ccaagggcagccgcgccatctggtatatgtggctaggggctagatttctagagttcgaag
Zika/HU/SI163851/Urine/MDH/NGS/2016    ccaagggcagccgcgccatctggtatatgtggctaggggctagatttctagagttcgaag
                                       ************************************************************

Zika/HU/SI163858SV/Saliva/MDH/2016     CCCTTGGATTCTTGAACGAGGATCACTGGATGGGGAGAGAGAACTCAGGAGGTGGTGTTG
Zika/HU/SI163858ESR/Eye-Swab/MDH/2016  cccttggattcttgaacgaggatcactggatggggagagagaactcaggaggtggtgttg
Zika/HU/SI163851U/Urine/MDH/2016       cccttggattcttgaacgaggatcactggatggggagagagaactcaggaggtggtgttg
Zika/HU/SI163851/Urine/MDH/NGS/2016    cccttggattcttgaacgaggatcactggatggggagagagaactcaggaggtggtgttg
                                       ************************************************************

Zika/HU/SI163858SV/Saliva/MDH/2016     AAGGGCTGGGATTACAAAGACTCGGATATGTCCTAGAAGAGATGAGTCGCATACCAGGAG
Zika/HU/SI163858ESR/Eye-Swab/MDH/2016  aagggctgggattacaaagactcggatatgtcctagaaagagatgagtcgcataccaggag
Zika/HU/SI163851U/Urine/MDH/2016       aagggctgggattacaaagactcggatatgtcctagaaagagatgagtcgcataccaggag
Zika/HU/SI163851/Urine/MDH/NGS/2016    aagggctgggattacaaagactcggatatgtcctagaaagagatgagtcgcataccaggag
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Zika/HU/SI163858SV/Saliva/MDH/2016     G
Zika/HU/SI163858ESR/Eye-Swab/MDH/2016  g
Zika/HU/SI163851U/Urine/MDH/2016       g
Zika/HU/SI163851/Urine/MDH/NGS/2016    g
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