Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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A Case of Fatal Zika Virus Infection with Secondary Non-Sexual Transmission: Supplementary Materials

Table of Contents

Additional clinical details: Patient 1

The patient was previously in good health with no chronic medical problems. He was born in Mexico and had immigrated to the United States in 2003. He was diagnosed with Stage IIB prostate cancer eight months earlier, had completed radiation therapy one month ago and was receiving anti-androgen therapy. He took no other medications and did not smoke, drink or use illicit drugs. Eight days prior to presentation, he had returned to the United States from a 3 week trip to the southwest coast of Mexico. During the trip, he went fishing in the ocean and had transient contact with water in a lagoon. He ate ceviche and soft-boiled turtle eggs. He recounted that since his departure, several people in the household in Mexico were told they had ZIKV infection.

Five days prior to admission, he experienced abdominal pain, sore throat and fever to 39.4^{o}C. The next day he developed redness and burning of his eyes and had 2 episodes of non-bloody diarrhea. The following day he had 3 more episodes of diarrhea and developed diffuse myalgia. He presented to the ED where he was alert and oriented. The temperature was 38.4^{o}C, pulse was 92 beats per minute, the blood pressure 112/56 mm Hg, the respiratory rate 17 breaths per minute, and the oxygen saturation 99% while breathing ambient air. Physical exam was notable for abdominal tenderness in the mid epigastrium without rebound or guarding. There was no rash, conjunctivitis or joint swelling. His initial laboratory testing was unremarkable except for chronic mild anemia (Table 1). The chest X-ray was unremarkable. He was observed for 24 hours and discharged.

The patient was subjectively improved with resolution of diarrhea until the evening prior to admission when shortness of breath developed. On the day of admission, he appeared unwell and was hypotensive and was referred to the ED. In the ED, the patient was in no distress and was alert and fully oriented. The temperature was 36.2^{o}C, the pulse was 119 beats per minute, the blood pressure 95/51 mm Hg, the respiratory rate 22 breaths per minute and the oxygen saturation 97% while on positive airway pressure ventilation. A tourniquet test was negative.

Laboratory testing revealed metabolic acidosis with an increased anion gap, respiratory alkalosis, elevated venous lactate, renal insufficiency, mild hypoglycemia, elevated transaminases, leukocytosis with 44% band forms, anemia and marked thrombocytopenia (Table 1). CT of chest, abdomen and pelvis was unrevealing. Empiric vancomycin and piperacillin-tazobactam were begun and doxycycline was added on day 2. A presumptive diagnosis of dengue shock syndrome was made.

Blood cultures drawn before antibiotics from both the ED visit and admission remained negative; *C. difficile* testing of stool and HIV PCR were both negative. Additional testing was ordered including *Leptospira* urine and blood PCR, dengue virus (DENV) PCR for all 4 serotypes, Chikungunya virus (CHIKV) PCR, *Leptospira* IgM, DENV and CHIKV IgM and IgG. Clinical deterioration progressed, requiring intubation with mechanical ventilation and initiation of continuous renal replacement therapy for oliguric renal failure and

worsening metabolic acidosis. Progressive hepatitis also developed. On day 4 of hospitalization, the patient's pressor requirements continued to increase and given his overall worsening clinical status, the family decided to withdraw care.

Both DENV and CHIKV PCR tests were negative as were CHIKV serologies. There was no autopsy performed. Next-generation sequencing of total RNA from a serum sample resulted in $1.1x10^6$ sequencing reads covering the entire ZIKV genome at a mean depth of more than 1,500-fold. The consensus sequence of the ZIKV strain from the index patient was most similar to ZIKV strains ZIKV/Aedes.sp/MEX/MEX_2-81/2016 (KX446950) and ZIKV/Aedes.sp/MEX/MEX_I-7/2016, (KX446951) (99.8% identity at the nucleotide level). The ZIKV strain contained 6 non-synonymous nucleotide polymorphisms compared to strain ZIKV/Aedes.sp/MEX/MEX_2-81/2016 (1 in pr, T55M; 2 in NS1: E265K, K326E; and 3 in NS5: C525R, I526T, S527P) and also 6 non-synonymous polymorphisms compared to strain ZIKV/Aedes.sp/MEX/MEX_I-7/2016 (1 in NS1: K326E; 1 in NS2A: V117A; and 4 in NS5: R384K, C525R, I526T, S527P). Novel protein polymorphisms were found in NS1 (K326E) and NS5 (I526T) proteins which have not been previously reported.

Additional clinical details: Patient 2

After Patient 1 expired the family was interviewed again. Patient 1 and his wife had visited Mexico together, but she had not experienced any symptoms. On his release from the ED, the rest of the family visited and congregated in the backyard where multiple family members reported experiencing mosquito bites.

Patient 2 reported developing symptoms of conjunctivitis, subjective fevers, myalgia and a facial maculopapular rash beginning 5 days after Patient 1 expired. The rash became generalized but resolved within 7 days although residual conjunctival hyperemia was present at day 11. Samples were obtained from Patient 2 at days 7 and 11 of illness. PCR of day 7 urine was positive for ZIKV but day 7 serum PCR was negative. Blood, urine, saliva and conjunctival specimens from day 11 were all negative by PCR (Table 2). All other routine laboratory testing was normal except for a slight elevation of the serum ALT that was previously normal.

Methods:

Total RNA was extracted from 200 μl of serum using the QIAamp MinElute Virus Spin Kit (Qiagen). cDNA sequencing libraries were prepared with the KAPA Stranded RNA-Seq Kit (KAPA Biosciences) and sequenced on a NextSeq 500 sequencer (Illumina). $3.6x10^7$ total paired-end sequencing reads were obtained. >1,500-fold mean coverage of the ZIKV genome was obtained.

Urine and serum PCR for ZIKV were performed with the Trioplex Real-Time RT-PCR assay as approved by the Centers for Disease Control (1). Zika MAC-ELISA for IgM against ZIKV was performed by the Centers for Disease Control (2).

Additional discussion

A patient with ZIKV infection was admitted to University of Utah Health Care with refractory hypotension progressing to multi-organ failure and death. This is the first ZIKV-related death reported in the continental United States. The patient was elderly and had recently received treatment for localized prostate cancer with radiation and anti-androgen therapy but was otherwise not systemically immunocompromised. His performance status prior to illness was excellent and he had no history of any chronic illness.

To date there have been confirmed reports of nine patients who died with ZIKV infection, of whom most had underlying malignancy, chronic co-morbidities or immunocompromising conditions (3-7). In the present case, superimposed or antecedent bacterial infection was not documented, and no other cause of the progressive fatal illness was identified. Further, the level of viremia was extremely high; approximately 2 X 10^8 ZIKV genome copies/ml. While the degree of viremia in most fatal ZIKV cases has not been established, a recent study reported > 1 x 10^9 copies/ml in the blood of a fatal case from Suriname (6). Whether high level viremia is associated

with severe disease and typical of fatal ZIKV cases will be important to determine in the future.

It is possible that Patient 1 had a specific type of underlying immunodeficiency predisposing to severe ZIKV infection but we consider this less likely since he had no history of severe or recurrent infections in the past. Further, the patient's laboratory test results were consistent with prior infection with a related flavivirus, DENV. The patient's family denied prior illness consistent with a severe dengue-associated syndrome, suggesting that the patient did not have a specific susceptibility to severe flavivirus infection. Nevertheless, the possibility of an acquired immunodeficiency cannot be excluded. Similarly, radiation therapy and androgen blockade may have played a role in enhancing ZIKV virus pathogenicity. In this regard, it is of interest that ZIKV persists in seminal fluid after clearance of viremia and may reach levels exceeding those in blood (8, 9), suggesting that cells in the male reproductive system may provide a milieu particularly suitable for ZIKV persistence and replication. It is possible that radiation therapy may have enhanced ZIKV replication in irradiated tissues and this may be a suitable area for further research. Recently, antibody dependent enhancement of ZIKV infection by cross-reacting DENV antibodies has been demonstrated in vitro (10), suggesting that previous dengue infection may also have contributed to the atypically severe ZIKV infection and high viremia in this patient.

The sequence of the ZIKV genome from Patient 1 showed highest sequence identity to a strain isolated from a mosquito trapped in Southwest Mexico (11). There was limited divergence from similar strains although there were several non-synonymous variants in the coding sequences. The novel polymorphisms are predicted to result in a substitution of threonine for isoleucine in the NS5 (polymerase) gene and lysine for glutamate in NS1. These changes are unlikely to represent a virulence determinant solely responsible for the atypically severe index case since the secondary case pursued a typical benign course. Nevertheless, the NS5 protein has been shown to target human STAT2 to inhibit Type I interferon signaling (12), and flavivirus NS1 proteins play roles in replication and immune evasion (13), leaving open the possibility that this strain may exhibit enhanced virulence.

A contact of Patient 1 with no identifiable risk factors for ZIKV infection developed a clinical syndrome compatible with ZIKV infection, detectable ZIKV RNA in urine and a positive ZIKV IgM serology. In the absence of any clear percutaneous or mucosal exposure, transmission may have occurred through inadvertent inoculation through skin or mucosal epithelium. In addition to the absence of *Aedes albopictus* or *aegyptii* mosquitoes in the area, transmission via mosquitoes appears unlikely for additional reasons. Patient 1 was not outdoors where mosquitoes could have fed on him until two days prior to admission. While Patient 2 was simultaneously present, there was no contact with Patient 1 again until admission to the hospital. Thus mechanical

transmission would have to have occurred without an intervening period of viral amplification in the mosquito.

Table 1. Laboratory values - Patient 1.

		3 days prior to admission	Day 1 of Admission	Day 4 of admission
Sodium	Ref Range: 136-144 mmol/L	136	132	151
Potassium	Ref Range: 3.3-5.0 mmol/L	4.1	4.5	3.6
Chloride	Ref Range: 102-110 mmol/L	104	100	104
Carbon Dioxide	Ref Range: 20-26 mmol/L	23	15	11
BUN	Ref Range: 8-24 mg/dL	12	49	54
Creatinine	Ref Range: 0.72-1.25 mg/dL	1.11	3.82	4.69

Glucose	Ref Range: 64-128 mg/dL	112	53	85
Calcium	Ref Range: 8.4-10.5 mg/dL	9.0	8.0	7.7
Total Protein	Ref Range: 6.5-8.4 g/dL	7.4	6.0	5.3
Albumin	Ref Range: 3.5-5.0 g/dL	4.0	3.2	3.6
Total Bilirubin	Ref Range: 0.2-1.4 mg/dL	1.0	0.9	1.7
Alkaline Phosphatase	Ref Range: 38-126 U/L	84	382	820
AST	Ref Range: 16-40 U/L	19	130	3847
ALT	Ref Range: 5-60 U/L	12	22	757
Anion Gap	Ref Range: 8-14 mmol/L	9	17	36

Lactate	Ref Range: 0.7-2.1 mmol/L	1.9	7.0	21
WBC	Ref Range: 4.30-11.30 k/uL	5.00	15.95	17.38
RBC	Ref Range: 4.70-6.14 M/uL	4.56	4.73	3.23
HGB	Ref Range: 14.8-17.8 g/dL	12.6	13.5	9.2
HCT	Ref Range: 44.2-53.0 %	40.5	41.9	28.6
MCV	Ref Range: 81.2-96.6 fL	88.8	88.6	88.5
MCH	Ref Range: 25.8-33.1 pg	27.6	28.5	28.5
MCHC	Ref Range: 31.9-35.2 g/dL	31.1	32.2	32.2

Platelet	Ref Range: 159-439 k/uL	139	33	11
Polymorpho-nuclear	Ref Range: 44-76 %	84	50	
Band Manual	Ref Range: 0-5 %	7	44	
Lymphocyte	Ref Range: 15-43 %	2	4	
Monocyte	Ref Range: 2-8 %	8	2	
pH, Urine	Ref Range: 5.5-7.5	5.5	5.0	
UA Appearance		Clear	Cloudy	
UA Bilirubin	Ref Range: Negative	Negative	Negative	
UA Blood		Moderate	Moderate	
UA Color		Yellow	Amber	
UA Glucose	Ref Range: Negative	Negative	Negative	
UA Ketones	Ref Range:	Negative	Negative	

	Negative			
UA Leukocyte Esterase	Ref Range: Negative	Negative	Negative	
UA Nitrite	Ref Range: Negative	Negative	Negative	
UA Protein	Ref Range: Negative	Trace	100	
UA Specific Gravity	Ref Range: 1.003-1.030	1.020	1.020	
UA Squamous Epithelial Cells	Ref Range: 0-5/HPF		2	
UA Urobilinogen	Ref Range: <2.0 mg/dL	0.2	Negative	

Organism	PCR	IgM	IgG
Patient 1 serum			
ZIKV	+	ND	ND
DENV	-	indeterminate	+
CHIKV	-	-	-
Patient 2			
ZIKV (serum, day 7)	-	+	ND
ZIKV (urine, day 7)	+	ND	ND
ZIKV (serum day 11)	-	+	ND
ZIKV (urine day 11)	-	ND	ND

Table 2. Serology and PCR results for Patients 1 and 2. Serum from Patient 1 was obtained on day 2 of hospitalization. Serum and urine from Patient 2 were tested on days 7 and 11 after onset of symptoms.

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