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Electronic Supplementary Material

Rift Valley Fever Virus and Yellow Fever Virus in Urine: A Potential Source of Infection

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Supplementary Materials and Methods

Patients and data collection

The clinical manifestations for the five yellow fever virus (YFV)-infected patients YF-BJ1, YF-BJ3, YF-BJ4, YF-BJ5 (Chen C. *et al*., 2018) , and YF-SH1 (Ling *et al*., 2016) and the one Rift Valley fever virus (RVFV)-infected patient RF-1 (Liu *et al*., 2017) included in the current study have been previously reported. Timelines of quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis and virus isolations for the five YFV-infected and one RVFV-infected patients are shown in Supplementary Fig. 1. Only patient YF-BJ5 had been previously vaccinated with the attenuated YFV vaccine, which occurred in Namibia 10 months prior to disease onset. Urine and serum samples of the patients were collected at various time points after disease onset (Supplementary Table S1).

RNA extraction

Viral RNA from the clinical specimens and cultured samples were extracted using a MagaBio plus Virus RNA Purification Kit (Automatic Nucleic Acid Purification System NPA-32+, BIOER, China) as previously described

(Yang *et al*., 2017).

Virus isolation

C6/36 and Vero cells (ATCC) were grown to 90% confluence in Dulbecco's modified Eagle's media (DMEM) (Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS) (GIBICO, USA) and 100 IU penicillin-streptomycin (Solarbio, China) and then washed three times with PBS buffer. Sera or urine samples from the YFV-infected or RVFV-infected patients were then inoculated onto the Vero or C6/36 cells. Supernatants were collected every 3 days after inoculation for detection of virus by qRT-PCR. After sampling at each time point, an equal volume of fresh supplemented DMEM was added to the cultures.

qRT-PCR

The clinical or culture samples from the YFV-infected or RVFV-infected patients were analyzed by qRT-PCR using Mabsky Bio-tech Co., Ltd. (China) the detection kits and an ABI QuantStudio 7 Real-Time cycler (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions. The sample was considered negative if the Ct value was greater than 37 or undetectable.

Next generation sequencing and phylogenetic analysis

YFV and RVFV viral RNA extracted from cell culture supernatants were fragmented using RNase H. The RNA fragments were then processed by end repairing, A-tailing, adapter ligation, DNA size-selection, circularization, and DNA nanoball formation according to in-house standard procedures for BGI library construction for BGI Seq500 (BGI-Shenzhen, China). DNA libraries with 300-bp inserts were sequenced using single-end 100-bp mode (SE100) on an BGI Seq500 Sequencer. Approximately 3 Gb was sequenced for each sample to obtain the whole-genome sequences of the viruses. Viral genome assemblies were done using an in-house script pipeline based on YFV Angola71 strain (GenBank No. AY968064.1) or RVFV Kakamas strain isolated from sheep in South Africa in 2009 (GenBank Nos. JQ068144, JQ068143, and JQ068142). Multiple sequence alignments were done using MUSCLE (Edgar, 2004). Phylogenetic analyses were done using the Maximum Likelihood method with 1000 replicates in Molecular Evolutionary Genetics Analysis version 6.0 (MEGA 6) (Tamura *et al*., 2013). Nucleotide sequence similarity among the isolates was analyzed using BioEdit version 7.0.4 software (Hall, 1999).

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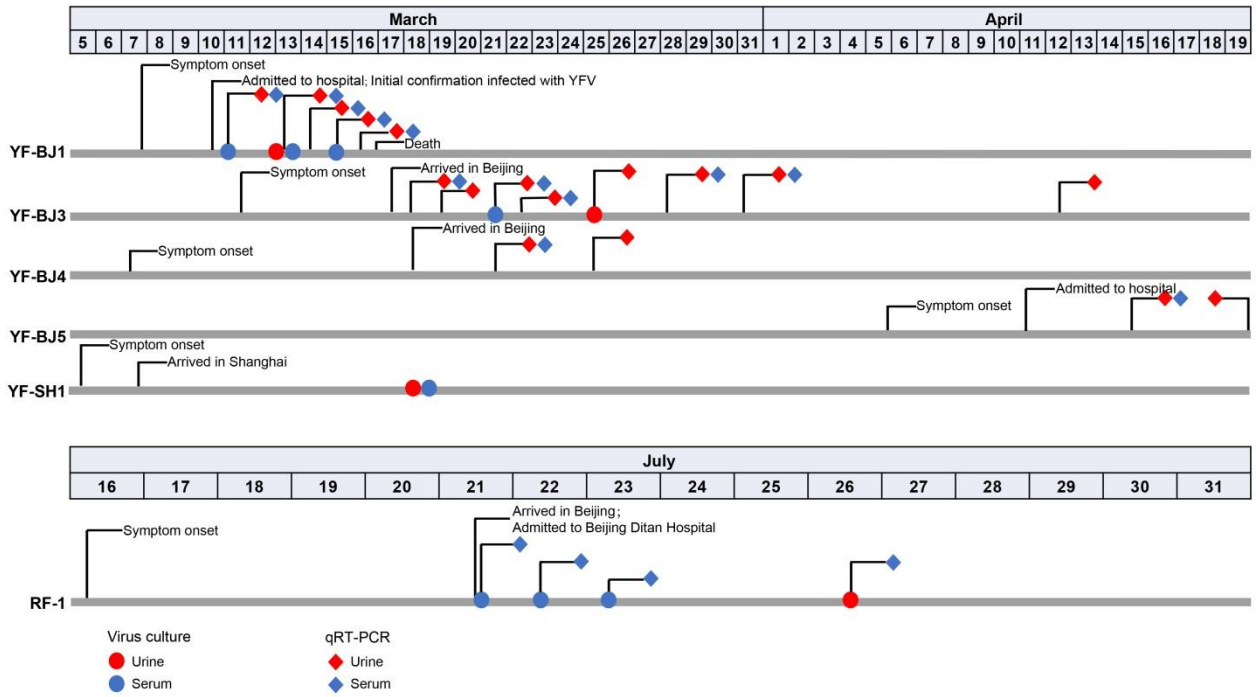

Fig. S1 Clinical timelines of yellow fever virus (YFV)-infected and Rift Valley fever virus (RVFV)-infected patients. Solid red and blue circles indicate the urine and serum samples, respectively, selected for infectious virus culture. Red and blue blocks indicate the urine and serum samples, respectively, used for virus detection with qRT-PCR analysis. For patient RF-1, the Ct values for detection of virus at the different time points have been previously reported (Liu *et al.*, 2017).

Table S1 Clinical information and viral load of the yellow fever virus (YFV)-infected and Rift Valley fever virus (RVFV)-infected patients

Patient ID	Sample collection time (days after disease onset)	Vaccination	Gender	Age (y)	Symptom severity	Ct value	
						Serum	Urine
				YFV			
YF-BJ1	4	No	M	32	Severe	**21**†	Neg
YF-BJ1	6					28†	28†
YF-BJ1	7					31	35
YF-BJ1	8					32†	33.5
YF-BJ1	9					30	34.42
YF-SH1	13	No	M	44	Mild	ND†	**ND**†
YF-BJ3	7	No	M	45	Severe	39.38	28.1
YF-BJ3	8					ND	25.6
YF-BJ3	10					27.9†	25.32
YF-BJ3	11					Neg	33.35
YF-BJ3	14					ND	**32.85**†
YF-BJ3	17					Neg	31.66
YF-BJ3	20					Neg	34
YF-BJ3	32					ND	39.83
YF-BJ4	15	No	M	51	Mild	Neg	30.89
YF-BJ4	19					ND	35.31
YF-BJ5	10	Yes	M	29	Mild	23.84	20.76
YF-BJ5	14					ND	34.23
				RVFV			
RF-1	6	−	M	45	Severe	**ND**†*	ND
RF-1	7					**28.7**†*	ND
RF-1	8					**31.4**†*	ND
RF-1	11					Pos*	**ND**†

Note: The amount of YFV and RVFV in serum and urine samples was quantified by quantitative reverse transcription polymerase chain reaction (qRT-PCR). For serum samples from patient RF-1, the Ct values at different times have been previously reported (Liu *et al*., 2017). ND, not done; Neg, negative by qRT-PCR; Pos, positive by qRT-PCR with unclear Ct value. The samples were considered as negative if the Ct value was greater than 37 or undefined. Black and bold Ct values indicate that infectious virus was successfully isolated but not sequenced. Green and bold Ct values indicate that infectious virus was successfully isolated and sequenced. Patient YF-BJ5 was vaccinated in Namibia 10 months prior to the onset of disease. −, no vaccine available; †, samples were used for virus culture

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Table S2 Genetic identity of each gene for the RVFV isolates

			S gene		
Source			**Serum**		**Urine**
	Sample	RF-1-D6	RF-1-D7	RF-1-D8	RF-1-D11
Serum	RF-1-D6	1	0.999	1	1
	RF-1-D7	0.999	1	0.999	0.999
	RF-1-D8	1	0.999	1	1
Urine	RF-1-D11	1	0.999	1	1
			M gene		
Source			**Serum**		**Urine**
	Sample	RF-1-D6	RF-1-D7	RF-1-D8	RF-1-D11
Serum	RF-1-D6	1	0.998	0.999	0.998
	RF-1-D7	0.998	1	0.998	0.999
	RF-1-D8	0.999	0.998	1	0.998
Urine	RF-1-D11	0.998	0.999	0.998	1
			L gene		
Source			**Serum**		**Urine**
	Sample	RF-1-D6	RF-1-D7	RF-1-D8	RF-1-D11
Serum	RF-1-D6	1	0.999	0.999	0.999
	RF-1-D7	0.999	1	0.998	0.998
	RF-1-D8	0.999	0.998	1	0.998
Urine	RF-1-D11	0.999	0.998	0.998	1

Note: RVFV genome is comprised of large (*L*), medium (*M*), and small (*S*) gene sequences. RF-1-D6, RF-1-D7, RF-1-D8, and RF-1-D11 indicate the strains isolated from patient RF-1 at 6, 7, 8, and 11 days after disease onset, respectively.