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LETTER

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

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Dear Editor,

In recent years, the incidence of human infections caused by emerging or re-emerging pathogens has rapidly increased. Diseases that were once regional now have the ability to spread globally in a short amount of time and pose a wider threat to public health (Weaver *et al.* 2018). Yellow fever virus (YFV, family *Flaviviridae,* genus *Flavivirus*) is a mosquito-borne flavivirus that causes yellow fever in humans and has been endemic in Africa and Latin America for many years (Domingo *et al*. 2018). The most recent large-scale outbreak of YFV occurred in Brazil in which the mortality rate as of February 28, 2018 is 32.78% (WHO 2018). Rift Valley fever virus (RVFV, family *Bunyaviridae,* genus *Phlebovirus*) is another mosquito-borne virus and primarily circulates in Africa and the Middle East, and in recent years in Europe (Mansfield *et al.* 2015). During the initial stage of infection, most patients infected with YFV or RVFV present nonspecific symptoms such as fever, headache, and vomiting, which often lead to a misdiagnosis (Mansfield *et al*. 2015; Domingo *et al.* 2018). The cases of YFV and RVFV in China were first reported in March and July 2016, respectively, in travelers returning from Angola (Chen *et al.* 2016; Liu *et al*. 2017).

Humans infected with RVFV or YFV can develop viremia during the acute phase of the disease. Currently, laboratory diagnosis of YFV (Domingo *et al*. 2018) and RVFV (Mansfield *et al*. 2015) infections are mainly performed using sera tested by quantitative reverse transcription polymerase chain reaction (qRT-PCR) or immunological methods such as enzyme-linked immunosorbent assay (ELISA). YFV (Domingo *et al.* 2018) and RVFV (Mansfield *et al*. 2015) can be detected in human kidneys or kidney fibroblasts and cause pathological changes in the kidneys of patients with severe disease. Recent studies have shown that YFV may be detected in urine samples and persists in the blood (Reusken *et al.* 2017; Barbosa *et al*. 2018; Domingo *et al*. 2018) and that viable YFV can be isolated from urine samples (Barbosa *et al*. 2018). In contrast, RVFV has been detected in urine

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samples using qRT-PCR (Haneche *et al.* 2016), but the isolation of live virus has not been reported.

Here, we report results from a comparative analysis of the detection window for YFV and RVFV in patients' sera and urine samples. Moreover, infectious YFV and RVFV were isolated from clinical samples, verified by sequencing, and whole-genome sequences were compared. Between March 8 and April 10, 2016, a total of 11 YFV cases were imported into China (Wang *et al.* 2016). On July 21, 2016, the first imported case of RVFV was reported in China (Liu *et al.* 2017). Serum and urine samples were collected at different time points after disease onset from five of the YFV patients infected with a West Africa II genotype of YFV (patients YF-BJ1, YF-SH1, YF-BJ3, YF-BJ4, and YF-BJ5) (Ling *et al.* 2016; Chen *et al.* 2018) and the RVFV patient infected with an E lineage RVFV (patient RF-1) (Supplementary Fig. S1, Supplementary Table S1). Of the five YFV-infected patients, only YF-BJ5 was vaccinated with the attenuated YFV vaccine with the vaccination occurring in Namibia 10 months prior to disease onset (Song *et al.* 2018) (Supplementary Table S1). Except for the death of YF-BJ1, the only fatality, the remaining patients with severe (YF-BJ3 and RF-1) or mild symptoms (YF-SH1, YF-BJ4, and YF-BJ5) were all recovered. The detection window for YFV in serum and urine was analyzed first by qRT-PCR (Supplementary Materials and Methods, Fig. S1A–S1D; Supplementary Table S1). In patient YF-BJ1, YFV was detected at comparable levels in all samples (Fig. 1A; Supplementary Table S1). For patient YF-BJ3, serum samples were positive until 10 days after disease onset (dao), whereas YFV in urine persisted until 32 dao (Fig. 1B; Supplementary Table S1), which was longer than other reports on natural YFV infections (Reusken *et al.* 2017; Barbosa *et al.* 2018). In addition, following vaccination with the attenuated vaccine strain YFV-17D, RNA was detected up to 198 days in urine post vaccination (Martins *et al.* 2013). In patients YF-BJ4 and YF-BJ5, the detection windows were also longer for urine compared to that for sera (Fig. 1C, 1D; Supplementary Table S1). For patient RF-1 (Liu *et al.* 2017), RVFV was detected in both sera and urine (Supplementary Table S1). As indicated above, our results were consistent with previous findings (Haneche *et al.* 2016; Reusken *et al.* 2017), suggesting that urine may be used as an appropriate clinical specimen for the detection of YFV and RVFV and may prove useful for instances when blood samples are not available.

We then attempted to isolate infectious virus from the urine and sera of patients YF-BJ1, YF-SH1, YF-BJ3, and RF-1. Whole-genome sequences of the isolates were obtained by next generation sequencing (NGS) and used for strain confirmation (Supplementary Materials and Methods; Supplementary Table S1). For patient YF-BJ1, viable virus was successfully recovered only from serum collected on 4 dao and the isolate was sequenced (YFV-BJ01/2016, GenBank No. KY495641). YFV-BJ01/2016 shared 99.5% nucleotide identity to the full-genome sequence of strain CNYF01/2016 (GenBank No. KX268355), which was previously isolated from the sera of the same patient at 3 dao (Chen *et al.* 2016). For patient YF-SH1, virus was isolated from the urine sample collected on 13 dao (YFV-SH01/2016, GenBank No. MG589641) and the isolate shared 99.9% nucleotide identity to strain Chinese Shanghai01 (GenBank No. KU949599), which was previously isolated from the sera of the same patient (Ling *et al.* 2016). Phylogenetic analysis of the whole-genome sequences showed that both strains YFV-BJ01/2016 and YFV-SH01/2016 belonged to the Angola genotype, while vaccine strains 17D (GenBank No. KF769015 and GenBank No. NC_002031) belonged to the West Africa II genotype (Fig. 1E). Furthermore, strains YFV-BJ01/2016 and YFV-SH01/2016 possessed only 74% nucleotide identity to full-genome sequences of 17D strains (KF769015 and NC_002031), indicating a long evolutionary distance between the prevalent circulating viruses and the vaccine strain. For patient YF-BJ3, infectious YFV was isolated in urine collected on 14 dao, but no viable virus was obtained from sera after 10 dao (Supplementary Table S1).

Viable RVFV was successfully isolated from the urine sample collected on 11 dao (GenBank Nos. MG649053, MG649057, and MG649061), consistent with the positive results from the serum samples collected at 6, 7, and 8 dao (GenBank Nos. MG649050-52, MG649054-56, and MG649058-60). Nucleotide identity was 99.8%–100% for the *S, M*, and *L* gene segments among four strains isolated from sera and urine (Supplementary Table S2). The genomic heterogeneity of YFV or RVFV from the same patient may have been due to intra-host evolution, as described previously (Chen *et al.* 2018). The *S, M*, and *L* gene segments of the isolates from sera clustered closely with the virus from urine and fell into Group E in the phylogenetic tree. The *L* gene results are shown in Fig. 1F. The sequence and assembly data of strains from patients YF-BJ1, YF-SH1, and RF-1 are available from the China National GeneBank (CNGB) Nucleotide Sequence Archive (CNSA; https://db.cngb.org/cnsa/; accession number CNP0000083).

In the current study, YFV and RVFV were detected in and isolated from the urine of infected patients. In addition, several arboviruses, such as West Nile virus (WNV), dengue virus, and Zika virus (ZIKV), have been previously isolated from the urine of patients (Barzon *et al.* 2013; Andries *et al.* 2015; Zhang *et al.* 2016; Barbosa *et al.* 2018). Therefore, if urine specimens from YFV-infected, RVFV-infected, or other arboviruses-infected patients are

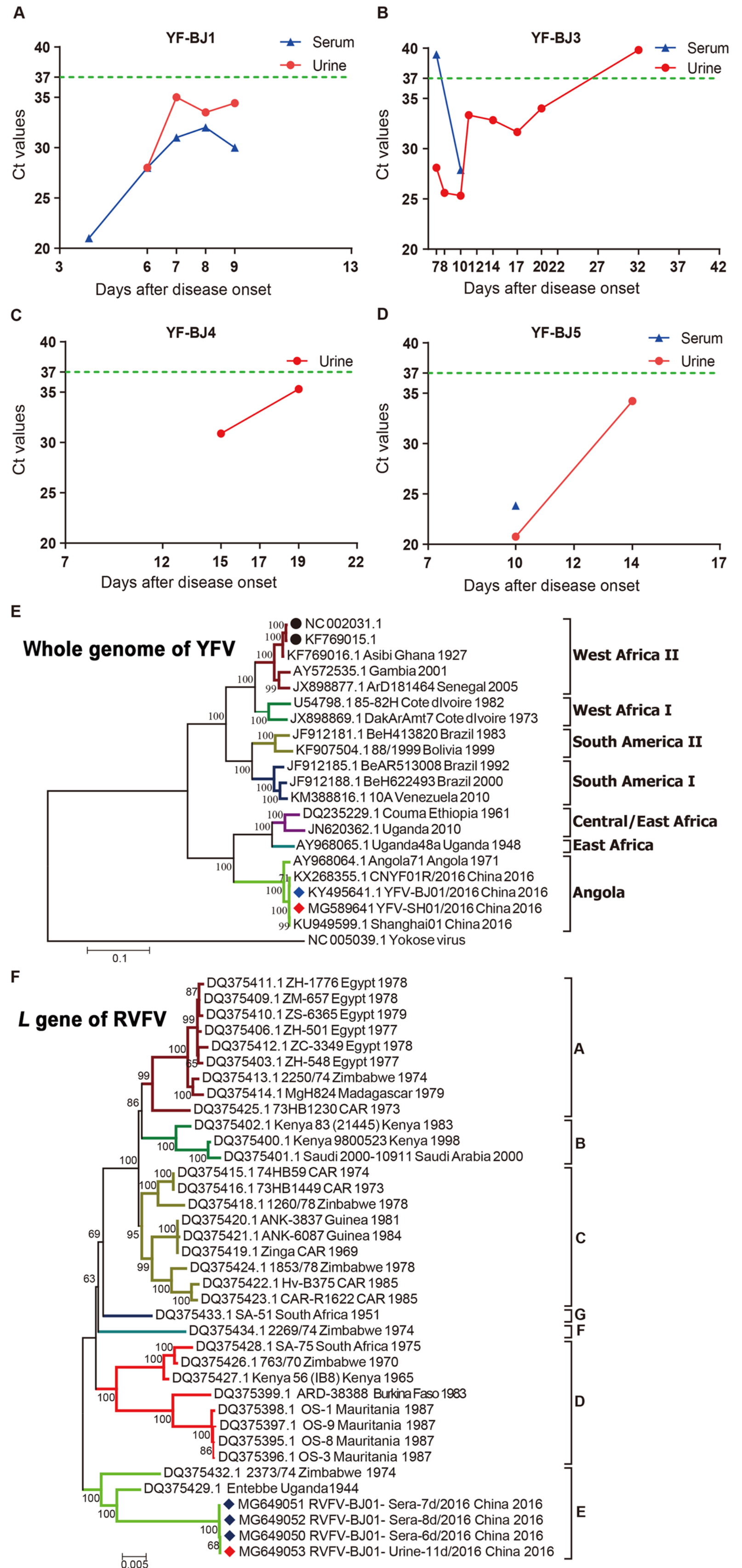

Fig. 1 **A**–**D** Detection of yellow fever virus (YFV) in serum and urine specimens from YFV-infected patients (YF-BJ1, YF-BJ3, YF-BJ4, and YF-BJ5) by quantitative reverse transcription polymerase chain reaction (qRT-PCR). The results from sera and urine are indicated in blue and red, respectively. The green dotted line represents the detection threshold by qRT-PCR. The Ct values are shown in Supplementary Table S1. **E**–**F** Phylogeny of Rift Valley fever virus (RVFV) and YFV isolates from patient sera and urine. Nucleotide sequences for the whole genome of YFV (**E**) and *L* gene of RVFV (**F**) were inferred by the Maximum Likelihood method with 1000 replicates using Molecular Evolutionary Genetics Analysis version 6.0 (MEGA 6). Strains indicated with a red or blue diamond represent sequences obtained from urine and serum samples, respectively, from the current study. Strains indicated with solid black circles represent YFV vaccine 17D strains. Each branch in the phylogenetic tree was named as follows: GenBank accession number, strain name, country of isolation, and year of isolation.

not properly treated, there might be a potential for transmission by direct contact or by vectors. The detection of viable YFV and RVFV in urine samples implied that there may have been active viral replication occurring in the kidneys of patients resulting in potential renal pathogenicity caused by the viruses. Moreover, accumulating evidences from studies show that urine samples might provide extended window for the molecular diagnosis of flaviviruses in patients, including WNV, dengue virus, ZIKV, and YFV (Barzon *et al.* 2013; Andries *et al.* 2015; Gourinat *et al.* 2015; Zhang *et al.* 2016; Reusken *et al.* 2017; Barbosa *et al.* 2018). Hence, urine may be considered as an alternative non-invasive source of samples for clinical detection of YFV, RVFV, and other vector-borne pathogens, which should improve diagnosis, help with surveillance efforts against the spread of these viruses, and reduce the risk of secondary infections.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Animal and Human Rights Statement Informed consent was obtained from all patients for the collection and use of all clinical specimens. This article does not contain any studies with animal subjects performed by any of the authors.

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