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Diagnosis of Dengue in a returning traveler from Pakistan suspected of COVID-19, California, USA

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ABSTRACT

Dengue and COVID-19 cocirculation presents a diagnostic conundrum for physicians evaluating patients with acute febrile illnesses, both in endemic regions and among returning travelers. We present a case of a returning traveler from Pakistan who, following repeated negative SARS-CoV-2 tests, was found to have a Dengue virus serotype 2 infection.

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1. Case report

Dengue virus (DENV) is a re-emerging arbovirus transmitted by the bite of an infected female *Aedes aegypti* or *Aedes albopictus* mosquito (Simmons et al., 2012). Over 390 million estimated human DENV infections occur annually (Bhatt et al., 2013). Infections are endemic throughout tropical and subtropical areas with incidence the highest in South and Southeast Asia, Central and South America, and sub-Saharan Africa (Guzman et al., 2016). Infection with one of the four DENV serotypes can result in an acute febrile illness typically lasting 5 to 7 days and often accompanied by headache, retro-orbital pain, muscle and bone pain, and maculopapular rash (Waggoner et al., 2016). A subset of cases will progress to severe dengue, a life-threatening illness characterized by systemic vascular leakage, marked thrombocytopenia, bleeding, shock, and organ dysfunction (Guzman et al., 2016).

Distinguishing dengue from other febrile illnesses can be difficult due to overlapping clinical manifestations, and this has become especially problematic in the setting of the global coronavirus disease 2019 (COVID-19) pandemic. Infection with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) can result in fever, myalgias, and other nonspecific symptoms which may mimic dengue or malaria, presenting a diagnostic conundrum for physicians in endemic areas (Saddique et al., 2020; Wee et al., 2020). In addition,

cocirculation of DENV and SARS-CoV-2 raises the possibility of coinfection and enhanced severity of each illness (Nacher et al., 2020; Rana et al., 2021). Some data suggest that social distancing measures aimed at mitigating the spread of COVID-19 have actually increased the spread of DENV, as most infections occur in the residential setting (Lim et al., 2020).

Cocirculation of DENV and SARS-CoV-2 also presents a diagnostic conundrum for physicians evaluating patients with acute febrile illnesses returning from travel to dengue endemic areas. In the United States in 2020, 332 dengue cases were reported to the Centers for Disease Control and Prevention, 252 (74%) of which were travel associated and 80 (26%) of which were locally-transmitted cases (CDC, 2020). All locally-transmitted cases occurred in the states of Florida ($n = 70$) and Texas ($n = 10$) (CDC, 2020). Appropriate diagnostic tools are vital to making the right diagnosis in these cases. In the case of dengue, diagnosis typically relies on serologic demonstration of IgM antibodies in an acute phase serum sample, IgG serologic testing using paired acute and convalescent phase serum, or molecular assays such as real-time reverse transcription polymerase chain reaction (RT-PCR) (WHO, 2009). Accurate SARS-CoV-2 diagnosis relies on viral RNA detection by RT-PCR or other nucleic acid amplification tests (NAATs) (Bulterys et al., 2020).

In October 2020, a 30-year-old previously healthy woman presented to a Stanford telemedicine clinic appointment complaining of 5 days of fever (up to 102.6°F), fatigue, myalgias, ageusia, dysgeusia, itchy throat, frequent loose stools, abdominal cramping associated

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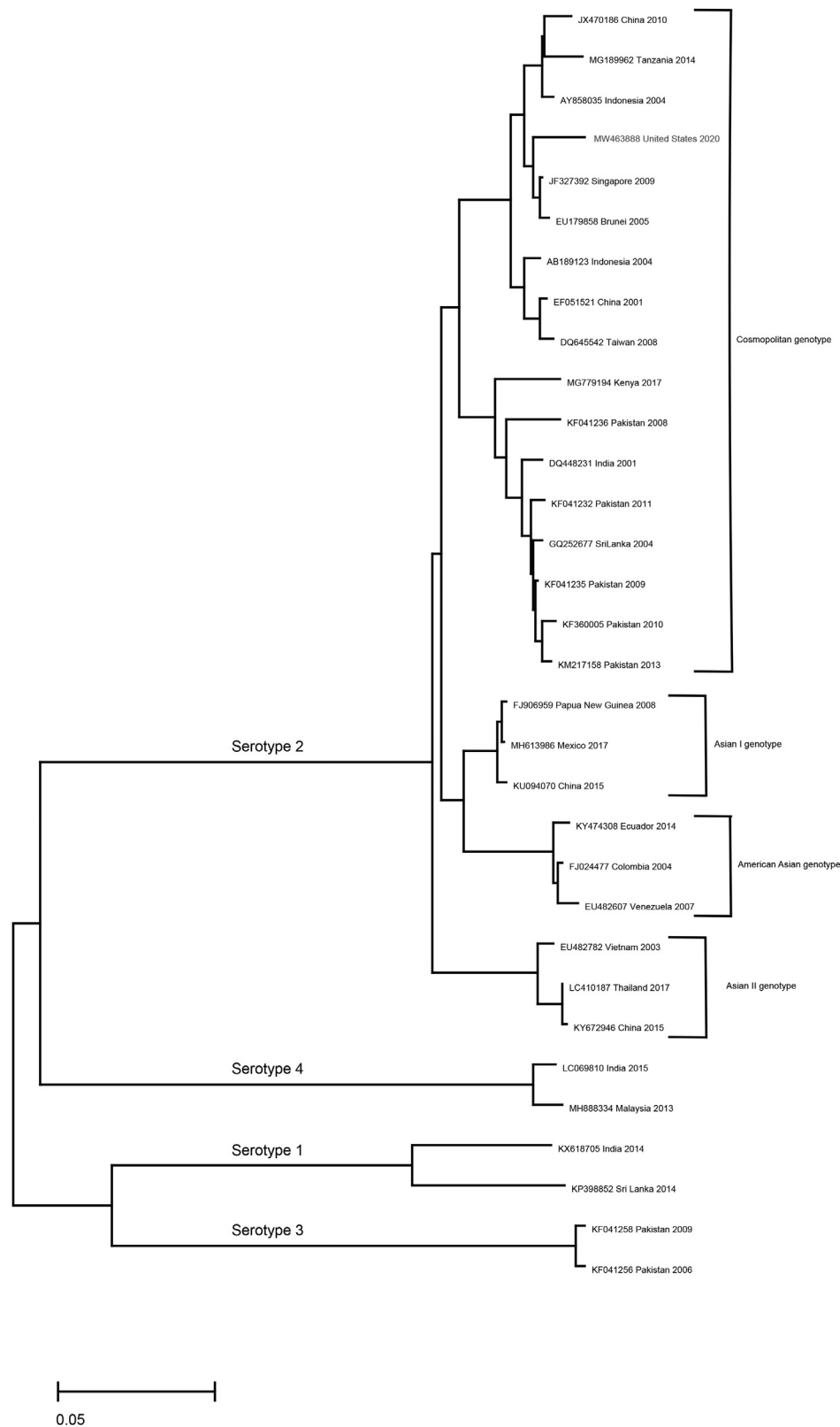


Fig. 1. Dengue virus phylogenetic tree, showing the relationship of the isolate from this case to other published DENV sequences. Multiple sequence alignment was performed with Clustal X2 (clustal.org) and the tree was rendered in Mega5.2 (megasoftware.net). The scale bar represents the number of substitutions per sequence position.

with meals, and anorexia. She did not have a rash, cough, or urinary symptoms. The day prior, she had also started having nausea and vomiting. The patient returned from a week-long trip to Pakistan 6 days prior (1 day before the onset of her symptoms). The day prior to her clinic appointment, she presented to an outside emergency

department and tested negative on SARS-CoV-2 NAAT, rapid influenza antigen test, mononucleosis heterophile screen, and urinalysis. Given that she was feeling generally unwell and complaining of symptoms suspicious for COVID-19, she was advised to present to the emergency department for repeat SARS-CoV-2 testing.

The patient presented to the Stanford emergency department later that day. Her temperature was 100.5°F on acetaminophen and ibuprofen. She disclosed that her mother, whom she had visited and cohabitated with in Pakistan, had developed similar symptoms recently and tested negative for SARS-CoV-2, and positive for DENV (testing method and serotype unknown). A physical exam revealed no rash, and a negative tourniquet test. Laboratory findings were significant for leukopenia (2.5 K/ μ L), absolute neutropenia (1.58 K/ μ L), lymphocytopenia (0.75 K/ μ L), monocytopenia (0.16 K/ μ L), undetectable eosinophils (0.00 K/ μ L), and elevated AST (54 U/L). She was negative for SARS-CoV-2 by an Emergency Use Authorized RT-PCR targeting the envelope gene (Bulterys et al., 2020). A chest X-ray showed no significant abnormalities. The patient was discharged home. Dengue virus RT-PCR targeting the 5' untranslated region and capsid gene of the DENV genome (Waggoner et al., 2016) was performed on plasma based on the patient's family history and returned positive, with a cycle threshold value of 19.3 (positive control = 27). The patient was notified by telephone of her positive DENV test result and instructed to rest, hydrate, and take antipyretics.

Serotype-specific, multiplex DENV RT-PCR (Waggoner et al., 2013) revealed that the etiologic virus in this case was dengue virus serotype 2, the predominant serotype in Pakistan (Tian et al., 2017). Serotype-specific primers D2_750F2(ATGGATGTCATCAGAAGGGCCTG) and D2_2300R2(ACAGACAGTGAGGTGCTACGTGAATT) were used to generate an amplicon spanning the glycoprotein M and part of the envelope gene. The amplicon was sequenced by Sanger at Elim Biopharmaceuticals (Hayward, CA). The sequence was submitted to GenBank as MW463888. The matching region from selected GenBank sequences were aligned using ClustalX2 (clustal.org) and a neighbor-joining tree was generated. MEGA5.2 (megasoftware.net) was used to create the phylogenetic tree. This revealed that the etiologic virus in this case clusters with other serotype 2 viruses of the cosmopolitan genotype (Fig. 1).

This case illustrates the importance of clinical awareness of dengue fever in returning travelers presenting with acute febrile illness during the COVID-19 pandemic. This is increasingly important given concerns that the COVID-19 pandemic may be amplifying the spread of dengue in endemic areas due to social distancing measures that increase residential exposure (Lim et al., 2020), and disruptions to surveillance as a result of reallocation of limited resources (Rabiu et al., 2021; Rana et al., 2021). Additional studies will be required to fully understand the impact of the COVID-19 pandemic on spread and diagnosis of dengue fever and other arboviral diseases, both in endemic areas such as Pakistan and among returning travelers.

Author contributions

PLB and BAP designed research. PLB, DS, MV, CH, MS, CC, MKS, and BAP performed research. PLB, DS, MKS, and BAP analyzed data. PLB, DS, MKS, and BAP wrote the paper.

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Declaration of competing interest

The authors declare no conflicts of interest.

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