

endurance horses from the United Arab Emirates that were collected for routine veterinary purposes; and 861 samples from 697 horses, 82 donkeys, and 82 mules in Spain. Because the reactivity of equid serum against MERS-CoV has not been investigated, we established a 2-stage algorithm for serologic testing that did not involve the determination of reactivity cutoff values. The screening stage involved testing of all serum samples by using a previously described ELISA with the spike protein S1-domain of MERS-CoV as the test antigen (4). The ELISA was adapted for use with horse serum by exchange of the secondary antibody. All serum samples reacted with low to medium OD values (range 0.0–0.55) (Figure, panel C). We then tested the 50 most reactive serum samples (optical density range 0.22–0.55) by using recombinant immunofluorescent and microneutralization assays (1). These assays are more specific than the ELISA assay and therefore can be used for confirmation. None of the tested serum samples showed reactivity in the recombinant immunofluorescent or microneutralization assays; this finding suggests that no previous exposure of equids to MERS-CoV has occurred in the United Arab Emirates and Spain.

Identifying all potential animal reservoirs is a critical step in controlling zoonotic diseases. Molecular data suggest that horses may be highly susceptible to MERS-CoV because of their high similarity in DPP-4 amino acids at positions critical for binding of the MERS-CoV spike protein (8). Our *in vitro* data confirm the susceptibility of primary horse cells, showing production not only of viral RNA but also of infectious virus progeny, which is a prerequisite for transmission. The lower replication observed in horse cells than in VeroB4 cells may be the result of a difference in the interferon competence of the cells; replication levels in horse cells are comparable to those in bat cells (6). Although we did not find evidence for equid infections with MERS-CoV in this study, the general susceptibility on the cell culture level suggests that equids from MERS-CoV–endemic areas, such as Africa and the Arabian Peninsula, should be further investigated for possible infection with MERS-CoV.

Acknowledgments

We thank Dagmar Hensel for excellent technical help and Matthias Lenk for horse cell lines. We also thank Victor Corman, Monika Eschbach-Bludau, Tobias Bleicker, and Sebastian Brünink for help with horse serum samples.

This study was supported by the European Commission under project ANTIGONE (contract no. 278976) and the German Centre for Infection Research.

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Autochthonous Dengue Fever Imported to England from Japan, 2014

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DOI: <http://dx.doi.org/10.3201/eid2101.141581>

To the Editor: Dengue fever, a mosquito-borne disease caused by dengue virus, can be asymptomatic or result in a variety of clinical manifestations, including fever, headache, myalgia, arthralgia, and rash (1). Severe cases can cause shock or severe hemorrhage (1). During the past 50 years, dengue has become a public health concern worldwide, rapidly spreading geographically, mainly in tropical and subtropical countries (1).

Before 2014, the most recent dengue outbreaks in Japan (located in the temperate zone), started in August 1942 on Kyusyu (the southernmost of Japan's 4 main islands) and recurred every summer until 1945 (2). Although no autochthonous dengue fever has been identified since then, a warning case occurred in August 2013, when dengue virus infection was diagnosed in a German traveler who returned from a 2-week trip to Japan (3). Reported here is an autochthonous dengue fever case imported from Japan to England in September 2014.

A 33-year-old international male student studying in England traveled to Japan and stayed with a friend in Tokyo during July–September 2014. In late August, an acute high fever (39.7°C), severe headache, retro-orbital pain, malaise, and loss of appetite developed. He did not have cough, sputum, or rhinorrhea. He returned to England 3 days after symptoms developed. On day 5 after symptom onset, the man noticed a scattered papular erythematous rash on the anterior chest wall. His symptoms had improved substantially. One week later, he experienced exacerbation of the same symptoms (again without cough, sputum, or rhinorrhea), which continued for a few days until he sought care 12 days after initial onset. He had no significant medical history or known allergies and had not traveled to any countries other than Japan.

On examination, the man appeared ill and had a high fever (38.5°C). His lungs were clear, but his pharynx was markedly swollen and erythematous with cervical lymphadenopathy. Despite his severe symptoms, results of laboratory tests showed no or only mild elevations: normal leukocyte count and differentials (6,700 cells/mm³ [reference 4,000–11,000] with neutrophils 60% and lymphocytes 26%) and slightly elevated C-reactive protein (0.9 mg/dL [reference 0.0–1.0 mg/dL]) and erythrocyte sedimentation rate (14 mm/h [reference 1–10 mm/h]). Liver enzyme levels were elevated: lactate dehydrogenase 623 IU/L (reference 125–243 IU/L), alanine aminotransferase 78 IU/L (reference 0–55 IU/L), aspartate aminotransferase 58 IU/L (reference 5–45 IU/L), and γ -glutamyl transpeptidase 81 IU/L (reference 12–64 IU/L), with normal total bilirubin (1.0 mg/dL [reference 0.2–1.2 mg/dL]). Mild hyponatremia (sodium 132 mmol/L [reference 135–145 mmol/L]) also was noted; otherwise the test results were unremarkable, including normal platelet count and coagulation panel. Serologic tests were positive for anti-dengue virus IgM but negative for anti-dengue virus IgG; viral RNA was not detected.

The patient was hydrated with intravenous fluid and discharged with acetaminophen as needed for fever and pain. He was instructed to avoid nonsteroidal antiinflammatory drugs because of possible bleeding risk. The sore throat resolved within 1 day, and his pain and fever were well controlled with acetaminophen.

This dengue outbreak in Japan started in Yoyogi Park, a public park in the Tokyo metropolitan area. From August

26, 2014, when the first case was identified, through October 30, a total of 160 autochthonous dengue fever infections occurred (4). The patient reported here stated that the house where he stayed in Japan is a 2-minute walk from Yoyogi Park and that he was bitten multiple times by mosquitoes in late August, although he did not enter the park or other implicated parks.

This outbreak has several possible causes. First, *Aedes albopictus* mosquitoes—1 of 2 main vector mosquitoes of dengue virus—are widespread in Japan. Although the other species, *A. aegypti*, has not been established in Japan, it was once identified at Tokyo International Airport (5). Second, the worldwide dengue fever incidence has increased exponentially during the past 50 years (1), and the number of cases imported to Japan has increased steadily since 1999 (6,7). Third, increased international travel, trade, and shipping, in addition to global warming, might have contributed to geographic expansion of the vectors.

Why this dengue fever outbreak started from Yoyogi Park remains unknown. One possibility is the popularity of Yoyogi Park, which holds \approx 100 events annually, including many international events. In July and August 2014, just before the first case was identified, Yoyogi Park hosted multiple festivals of countries in dengue-endemic regions, including Southeast Asia and South and Central America (<http://www.yoyogipark.info/ad2014/>). Dengue virus could have been spread from infected visitors by mosquitoes in the park. Any events that include persons from dengue-endemic regions and held where the vectors are prevalent could be the source of spread.

This case highlights the possible risk for a dengue outbreak in countries to which dengue is not endemic but where the vectors are present, and thus the potential exists for travelers to become infected. Physicians need to be aware of the possibility of dengue fever in patients returning from non-tropical/subtropical countries, obtain a full travel history, and keep apprised of the latest epidemic information.

Acknowledgment

I thank Masahiko Sakai for technical support.

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Genome Sequence of Enterovirus D68 from St. Louis, Missouri, USA

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To the Editor: During the current (2014) enterovirus/rhinovirus season in the United States, enterovirus D68 (EV-D68) is circulating at an unprecedented level. As of October 6, 2014, the Centers for Disease Control and Prevention (CDC) had confirmed 594 cases of EV-D68 infection in 43 states and the District of Columbia (<http://www.cdc.gov/non-polio-enterovirus/outbreaks/EV-D68-outbreaks.html>); the actual number of cases was undoubtedly much higher. In mid-August, hospitals in Missouri and Illinois noticed an increased number of patients with severe respiratory illness (1). We observed this pattern at St. Louis Children's Hospital in St. Louis, Missouri.

Resources for studying this virus are limited. Before the current season, only 7 whole-genome sequences and 5 additional complete coding sequences of the virus were available. Therefore, determining whether there are genomic elements associated with rapid spread or severe and unusual disease was not possible.

To address these limitations, we determined the complete coding sequence of 1 strain from St. Louis by using high-throughput sequencing of nucleic acid from a clinical sample. To evaluate the sequence diversity in EV-D68 strains circulating in the St. Louis metropolitan area, we also generated partial-genome sequences from 8 more EV-D68-positive clinical samples from St. Louis. During the

preparation of this article, CDC generated and submitted to GenBank 7 complete or nearly complete genome sequences from viruses obtained from the Midwest. We documented the diversity of the sequences of strains from St. Louis and compared them to publicly available sequences.

The methods are described in brief here and in more detail in the online Technical Appendix (<http://wwwnc.cdc.gov/EID/article/21/1/14-1605-Techapp1.pdf>). This study was conducted under a protocol approved by the Human Research Protection Office of Washington University in St. Louis.

Patients were categorized retrospectively as having mild, moderate, or severe disease if they had been discharged home from the emergency unit, admitted to general wards, or admitted to the pediatric intensive care unit, respectively. Residual material from a subset of nasopharyngeal specimens positive for rhinovirus/enterovirus (tested by the BioFire FilmArray Respiratory Panel [BioFire Diagnostics, Salt Lake City, UT, USA] at the Clinical Virology Laboratory, St. Louis Children's Hospital) was selected for high-throughput sequencing. Total nucleic acid was extracted from clinical samples by using NucliSENS easyMAG (bioMérieux, Marcy l'Etoile, France) and used to make dual-indexed sequencing libraries. Enterovirus/rhinovirus sequences were enriched by using a NimbleGen custom sequence capture reagent (Roche/NimbleGen, Madison, WI, USA), which as of February 2014 was selective for all complete enterovirus and rhinovirus genomes in GenBank. Sequence data were generated on an Illumina HiSeq 2500 (Illumina Inc., San Diego, CA, USA). Sequences were assembled with IDBA-UD (2) and manually improved. The most contiguous genome was annotated by using VIGOR (3). Publicly available sequences were downloaded and compared by using the National Institute of Allergy and Infectious Diseases Virus Pathogen Resource (<http://www.viprbrc.org>) (4). Variants were identified by using VarScan (5). The sequence was deposited in GenBank under accession no. KM881710, BioProject PRJNA263037.

For 14 of the 17 samples, high-throughput sequencing data were interpretable (online Technical Appendix Table); for the other 3 samples, the number of virus sequence reads was too low to distinguish them from sample cross-talk, which occurs during high-throughput sequencing analysis (6). Of the 14 typed samples, EV-D68 sequences were detected in 7 of 10 samples from patients with severe disease, 2 of 2 with moderate disease, and 0 of 2 with mild disease. The complete coding sequence was assembled from sample EV-D68_STL_2014_12. The most closely related genomes from previous seasons were Thailand, CU134, and CU171 (7) (Figure, panel A). Several of the genome sequences obtained from Missouri strains from this season, which had been sequenced by CDC, were very similar to this genome sequence. Comparison of the virus protein 1 sequence with