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GENOME SEQUENCES

Complete Genome Sequence of SARS-CoV-2 in a Tiger from a U.S. Zoological Collection

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ABSTRACT This report describes the identification and characterization of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a Malayan tiger in a U.S. zoo.

The 2019 novel coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (family *Coronaviridae*, genus *Coronavirus*), was first reported in Wuhan, China, in December 2019 (1). As of April 2020, the global pandemic has affected over 2.7 million people across 185 countries/regions (https://coronavirus.jhu.edu).

On 2 April 2020, diagnostic samples were collected from a Malayan tiger (*Panthera tigris* subsp. *jacksoni*) with a cough of 1 week's duration at the Wildlife Conservation Society's Bronx Zoo in New York City, New York. Nasal and oropharyngeal swabs, as well as aseptically collected tracheal wash samples, were obtained from the anesthetized tiger. Duplicate samples were submitted to two independent laboratories in the National Animal Health Laboratory Network (NAHLN) (https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/lab-info-services/nahln/ct_national_animal_health_laboratory_network), namely, the University of Illinois Veterinary Diagnostic Laboratory (UIUC-VDL) and Cornell University Animal Health Diagnostic Center and New York State Veterinary Diagnostic Laboratory (AHDC), for SARS-CoV-2 real-time reverse transcriptase PCR (rRT-PCR) testing.

On 3 April 2020, all samples were positive by SARS-CoV-2 rRT-PCR testing at both laboratories using assays targeting two different genes (the ADHC used the CDC N-target assay [2], and the UIUC-VDL used an in-house E-gene target). Samples were driven overnight from the UIUC-VDL to the U.S. Department of Agriculture (USDA) National Veterinary Services Laboratories (NVSL) to ensure rapid confirmatory testing and compliance with the World Organisation for Animal Health (OIE) reporting procedures. Results of rRT-PCR testing (CDC N-target assay) at the NVSL were consistent with results from the two NAHLN laboratories on 4 April 2020. Cycle threshold values from rRT-PCR assays with the nasal swab and tracheal wash samples were consistently lower (suggesting more viral target) than those from the oropharyngeal swab. All three testing laboratories generated sequence data independently from RNA extracted

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TABLE 1 Total number of reads obtained for each library preparation

Laboratory identifier	SRA library identifier	Sequencing platform	Total no. of reads for:	
			Read 1	Read 2
A	055378-20-1-1	MiSeq	361,802	361,802
A	055378-20-1-2	MiSeq	387,290	387,290
B	20-33862-2	iSeq	1,234,345	1,234,345
B	20-33862-2(2)	iSeq	1,109,354	1,109,354
B	20-33862-P1	MiSeq	1,144,066	1,144,066
B	20-33862-P2	MiSeq	1,223,544	1,223,544
C	20-010292-002trt	Ion S5	47,351	NA[a]

[a] NA, not applicable.

directly from the diagnostic samples. Sequence data were consistent across all laboratories and with available SARS-CoV-2 sequences from humans.

Full genome data (SARS-CoV-2/tiger/NY/040420/2020) were obtained using different partial-genome and whole-genome sequencing approaches. Laboratory A extracted RNA using the MagMAX pathogen RNA/DNA kit, prepared genomic libraries using the PrimalSeq (3) tiled amplicon method with the ARTIC nCoV-2019v2 primer scheme (https://github.com/artic-network/artic-ncov2019), and sequenced the libraries on an Illumina MiSeq system using 2 × 250-bp paired-end reads. Laboratory B extracted RNA using the BioSprint 96 one-for-all vet kit and used a combination of metagenomics, with sequence-independent single-primer amplification (SISPA) (4, 5), and targeted amplification using 23 pairs of primers designed in-house specifically for SARS-CoV-2. Libraries were prepared using the Illumina Nextera XT DNA library preparation kit and sequenced on an Illumina iSeq system using 2 × 150-bp paired-end reads and on a MiSeq system using 2 × 150-bp or 2 × 250-bp paired-end reads. Laboratory C extracted RNA with the MagMAX-96 viral RNA isolation kit, prepared random cDNA libraries using SISPA followed by the Ion Xpress Plus fragment library kit, and sequenced the libraries using the Ion Chef and Ion S5 systems. The total number of reads generated from each library preparation is shown in Table 1. The Illumina sequences were combined combined, and a reference-guided assembly was performed against the complete genome sequence for SARS-CoV-2 strain Wuhan-Hu-1 (GenBank accession number NC_045512.2). For data from laboratory A, amplification primers and low-quality bases were trimmed from mapped reads using iVar v. 1.1 with default settings (slide window size, 4 bp; minimum quality score, 20; minimum read length, 30 bp). BAM files from the two libraries were merged and a pileup file was generated using SAMtools v. 1.9, and iVar v. 1.1 was used to generate a consensus assembly with default parameters (minimum base quality, 20; plurality consensus; minimum depth, 1). For combined data, all FASTQ reads were aligned to the Wuhan-Hu-1 sequence (NC_045512.2) using bwa v. 0.7.17-r1188. Alignment was visually verified in Geneious v. 2020.0.4, the consensus was determined, and raw reads were aligned back to the consensus to confirm. The final consensus sequence length was 29,897 nucleotides (G+C content, 37%). A total of 6 nucleotide changes were observed between the Wuhan-Hu-1 sequence and the consensus sequence. Illumina and S5 reads were then aligned against the consensus sequence to verify the assembly. The Illumina coverage depth was 7,000×, with 99.98% coverage of the reference genome, and the Ion S5 coverage depth was 74×, with 99.5% coverage.

Because of the rapid actions and collaborative efforts of the Bronx Zoo, state and federal animal health authorities, the NAHLN, and the NVSL, this finding was confirmed within 2 days after sample collection, including generation of genomic sequence data from three independent laboratories. This finding should not be used to infer risk of SARS-CoV-2 infection in companion animals. The tiger is progressively recovering; an in-depth case report is in preparation.

Data availability. The consensus data for the SARS-CoV-2/tiger/NY/040420/2020 genome have been deposited in the GISAID database (accession number EPI_ISL_

420293) and GenBank (accession number MT365033 and BioProject accession number PRJNA627354). The version described in this paper is the first version.

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