Near-Full-Length Genome Sequences Representing an Event of Zooanthroponotic Transmission of SARS-CoV-2 Lineage B.1.189 in Mexico during 2020

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ABSTRACT
Here, we report three near-full-length genome sequences of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) obtained in Mexico City, Mexico, during the pandemic of coronavirus disease 19 (COVID-19) in 2020, representing a zooanthroponotic transmission event between humans and a dog. All three genomes belong to the B.1.189 lineage based on the pangolin classification.

Considered the biggest sanitary event of the century, the coronavirus disease (COVID-19) pandemic is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the Coronaviridae family within the Betacoronavirus genus. To date (8 May 2022), the cases and deaths produced by this virus have been 513,955,910 and 6,249,700, respectively. Mexico has been one of the countries with the highest number of deaths (324,334) during this pandemic (https://covid19.who.int/), representing 5.18% of the total mortality worldwide.

The remarkable genome plasticity displayed by SARS-CoV-2 (1) leads to the divergence of multiple phylogenetic clades and the consequent emergence of different viral variants of concern (2). Therefore, the control of this pandemic has represented a major challenge (3, 4).

Recent reports documented the zooanthroponotic spillover of variants of concern like Delta (cats, dogs, pumas, lions, and hamsters) and Omicron (white-tailed deer) in wild and domestic animals (5, 6). Thus, documented infections produced by human-to-animal transmission are increasing (7, 8).

Here, we report three near-full-length genome sequences of SARS-CoV-2 strains obtained from nasopharyngeal swab specimens recovered during a zooanthroponotic spillover event between humans and a dog in Mexico City, Mexico, in 2020. All sequences were classified as part of the pangolin lineage B.1.189 (Fig. 1). Interestingly, no changes were observed in the consensus sequence obtained from the dog, showing the apparent genetic stability of this lineage after infection in different species.

Viral isolation was performed in Vero cells (ATCC C1008). Subsequently, RNA from the three viral isolates was extracted using the high pure viral RNA kit (Roche), following the manufacturer’s protocol. Next-generation sequencing (NGS) of amplicons was conducted to obtain the SARS-CoV-2 sequences reported in this announcement. For this purpose, a set of 15 primers were developed to cover the genome of SARS-CoV-2 (Table 1). Reverse transcriptase PCR (RT-PCR) reactions were conducted using the SuperScript III one-step RT-PCR system with Platinum Taq DNA polymerase kit, following the manufacturer’s instructions. Libraries were prepared using the Nextera XT DNA library preparation kit.
following the manufacturer’s protocol. Sequencing and analyses were conducted on the MiSeq system (Illumina). Raw data of samples identified as hCoV-19/dog/Mexico/CPALB32021033/2020, hCoV-19/Mexico/CPALB32021034/2020, and hCoV-19/Mexico/CPALB32021035/2020 consisting of 3,348,413, 8,248,286, and 9,425,298 reads, respectively,
with an average read length of 200 bp were analyzed. All analyses were performed in CLC Genomics Workbench v11.0. The paired reads were quality trimmed using default parameters. Reads were then mapped to the reference strain sequence (GenBank accession number NC045512.2). Consensus sequences were obtained using default parameters and annotated based on a comparison with the reference strain. All work conducted in humans and animals was approved by bioethics committee Escuela Nacional de Medicina y Homeopatía (ENMH) number CBE/006/2020 on the project “Zoonosis Virales Emergentes en Tiempos de Circulación de COVID-19 en México.” The information from these events is useful for defining the potential role of dogs as reservoirs or intermediate hosts of SARS-CoV-2. In addition, future studies may help evaluate the possible differences in the transmission in animal species among SARS-CoV-2 lineages.

**Data availability.** Sequences are available in the Global Initiative on Sharing All Influenza Data (GISAID) database under the following accession numbers: EPI_ISL_11991713 (hCoV-19/dog/Mexico/CPALB32021033/2020), EPI_ISL_11988443 (hCoV-19/Mexico/CPALB32021034/2020), and EPI_ISL_11988444 (hCoV-19/Mexico/CPALB32021035/2020). The raw sequencing data of this project are available in the NCBI Sequence Read Archive (SRA) under the BioProject number PRJN827138.

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**TABLE 1 Sequencing considerations**

| Amplicon no. | Primer ID | Primer sequence (5’–3’ | Location in reference sequence | Size (bp) | Annealing temp (°C) |
|-------------|-----------|-------------------------|-------------------------------|-----------|-------------------|
| 1           | 1FCOVID   | GCC TTC CCA GGT ACG AAS CCA ACC | 15–1931 | 1,916 | 58 |
| 2           | 2FCOVID   | GAG CAG TTT CAA GAG TGC GAG | 1686–4148 | 2,280 | 56 |
| 3           | 3FCOVID   | GCA TTT GCA TGA GGT GCT GCT CG | 4046–6371 | 2,325 | 58 |
| 4           | 4FCOVID   | GCC AAT CTT CAT CCA GAT TCT GGC | 6008–8372 | 2,364 | 56 |
| 5           | 5FCOVID   | TGT CCT GGG CTG CCT CTG ACT TC | 8169–10209 | 2,040 | 58 |
| 6           | 6FCOVID   | CAG CAG TCT GGC AAG GGT TTG TTG | 81222–12261 | 2,239 | 56 |
| 7           | 7FCOVID   | CCA AAG CCC TCT ATC ACC TCA GCT G | 12078–14333 | 2,255 | 55 |
| 8           | 8FCOVID   | CGA GCT AGT CAC ATG TTG ACA CTG | 14195–16411 | 2,216 | 55 |
| 9           | 9FCOVID   | CGG TGA CAT CAC AAG GGT CAC CG | 16215–18466 | 2,251 | 58 |
| 10          | 10FCOVID  | CGG TGT GGA CAT TGC TGC TAC CAC TAA C | 18306–2099 | 1,793 | 57 |
| 11          | 11FCOVID  | GGTT GTG GTA CAT TGC TGC TAC CAC TAA C | 19845–22446 | 2,601 | 56 |
| 12          | 12FCOVID  | GGG TGA GGA CAT TGC TGC TAC CAC TAA C | 22332–24239 | 1,907 | 58 |
| 13          | 13FCOVID  | GCC ACC TTT GTC GAC AGA TGA AAT G | 24145–26353 | 2,208 | 55 |
| 14          | 14FCOVID  | GCC GCG TAA GGA TGG GTA GTG | 26192–28375 | 2,183 | 60 |
| 15          | 15FCOVID  | GCC CCC CCG ATT AGC TTT GGC TG | 28307–29798 | 1,491 | 60 |

*Description of multiple sets of primers developed in this study to conduct the NGS amplicon sequencing described in this study. The location of the primers corresponds to the nucleotide positions in the reference sequence of SARS-CoV-2 under the accession no. NC045512.2.*

*ID, identification.
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