Brief Report

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a Dog, Connecticut, February 2021

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Abstract: We report the first detection of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus from a dog in Connecticut during February 2021. Complete genome sequencing and phylogenetic analysis of the hCoV-19/USA/CT-CVMDL-Dog-1/2021 (CT_Dog/2021) virus were conducted to identify the origin and lineage of the virus. The CT_Dog/2021 virus belonged to the GH/B1.2. genetic lineage and was genetically close to SARS-CoV-2 identified from humans in the U.S. during the winter of 2020-2021. However, it was not related to other SARS-CoV-2 identified from companion animals in the U.S. It contained both D614G in spike and P323L in nsp12 substitutions which have become the dominant mutations in the United States. The continued sporadic detections of SARS-CoV-2 in companion animals warrant public health concerns about their potential to become a new reservoir species of SARS-CoV-2.

Keywords: SARS-CoV-2; Dog; Connecticut; Next-generation Sequencing; Phylogenetics

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel zoonotic coronavirus identified as the cause of coronavirus disease 2019 (COVID-19) that caused a pandemic in late 2019 [1]. Several animal species have been reported to be susceptible to SARS-CoV-2 infection either naturally (cats, dogs, lions, minks, tigers) or after experimental infection (cats, Egyptian fruit bats ferrets, hamsters, mice, primates, and tree-shrew) [2-4]. In the United States, two domestic cats were confirmed infection with SARS-CoV-2 during April 2020 which was the first report of SARS-CoV-2 infection in companion animals in the U.S. [5]. Since then, a total of 113 cases have been reported in cats (n = 67) and dogs (n = 46) in the U.S. as of March 9 2021 [5].

Here, we report the first natural infection case of SARS-CoV-2 in dogs in Connecticut. On February 12, 2021, a 3-months-old, female German Shepard dog was presented for postmortem examination at Connecticut Veterinary Medical Diagnostic Laboratory (CVMDL) due to sudden death with no signs of illness, as reported by the owner. The left lung was diffusely discolored purple and the heart had coalescing areas of pallor that extended into the myocardium. Most likely, the cause of death was due to acute cardiac failure. Four different types of specimens (nasal, oral rectal swabs and lung tissue) were submitted to molecular diagnostic laboratory at CVMDL. All tested specimens were positive for SARS-CoV-2 by real-time reverse transcription PCR (rRT-PCR) [6]. Subsequently the sample was confirmed positive for SARS-CoV-2 by the USDA National Veterinary Services Laboratories.

2. Materials and Methods

Complete genome sequencing and phylogenetic analysis of the hCoV-19/USA/CT-CVMDL-Dog-1/2021 (CT_Dog/2021) virus were conducted to identify the origin and lineage of the virus. We extracted viral RNA from the sample using the RNeasy Plus Mini Kit (Qiagen) and amplified genome by multiplex RT-PCR using the QIAseq SARS-CoV-2 Primer Panel (Qiagen). DNA library was generated using Swift 2S™ Turbo DNA Library Kits (Swift Biosciences) and sequenced using the iSeq100 platform with the 300 cycle i1 Reagents v2 (Illumina). Sequencing reads were trimmed and filtered using BBDuk (https://sourceforge.net/projects/bbmap) with minimum length 100bp and minimum quality score of 30. Quality-filtered reads were then mapped to the reference sequence (GenBank accession no. NC045512) by using minimap2 [7]. Complete genome sequence has been deposited in GISAID (accession number EPI_ISL_1241386). Genetic lineage of the sample was assigned using the PANGOLIN v2.0 (https://github.com/hCoV-2019/pangolin). For phylogenetic analysis, all complete sequences of SARS-CoV-2 from companion animals in the U.S. and additional 8-10 sequences belonging to same genetic lineages were retrieved from the GISAID database. We generated maximum-likelihood (ML) phylogeny using RAxML v8.2.4 and the general time-reversible nucleotide substitution model, with among-site rate variation modeled by using a gamma distributed rate heterogeneity and a proportion of invariable sites [8]. We generated bootstrap support values using 1,000 rapid bootstrap replicates. Amino acid variations were identified using the CoV-GLUE engine v1.1.107 (http://cov-glue.cvr.gla.ac.uk).

3. Results and Discussion

A total of 2,120,432 reads were assembled into a single consensus sequence with 100% coverage of the reference and high mean depth of coverage (10054.9). The genome sequence of CT-dog/2021 virus was assigned as B1.2. by PANGOLIN and GH by GISAID classification. BLAST search results in the GISAID database indicated that the virus shared >99.97% nucleotide identity with SARS-CoV-2 identified in the U.S. during the winter of 2020-2021 (Table 1).

Table 1. SARS-CoV-2 sharing the highest nucleotide identity found by BLAST search in GISAID database on March 14, 2021.

GISAID accession	Virus	Location	Collection date (yyyy-MM-dd)	Sequence identity
EPI_ISL_1137193	hCoV-19/USA/PA-MGEL-01496/2021	USA/Pennsylvania	2021-01-25	99.99%
EPI_ISL_853340	hCoV-19/USA/PA-MGEL-01148/2020	USA/Pennsylvania	2020-12-02	99.99%
EPI_ISL_1137240	hCoV-19/USA/PA-MGEL-00902/2020	USA/Pennsylvania	2020-10-30	99.98%
EPI_ISL_1202236	hCoV-19/USA/TX-HMH-MCoV-16526/2020	USA/Texas	2020-11-03	99.98%
EPI_ISL_1094242	hCoV-19/USA/FL-CDC-2-3847117/2021	USA/Florida	2021-01-20	99.98%
EPI_ISL_1080077	hCoV-19/USA/TX-HMH-MCoV-25439/2021	USA/Texas	2021-01-21	99.98%
EPI_ISL_1075269	hCoV-19/USA/TX-HMH-MCoV-20826/2021	USA/Texas	2021-01-02	99.98%
EPI_ISL_1049241	hCoV-19/USA/UT-UPHL-2102150865/2021	USA/Utah	2021-02-02	99.97%
EPI_ISL_783726	hCoV-19/USA/TX-HMH-MCoV-19267/2020	USA/Texas	2020-11-24	99.97%
EPI_ISL_1095270	hCoV-19/USA/AZ-CDC-2-3846423/2021	USA/Arizona	2021-01-14	99.97%

In the ML phylogeny, the CT_Dog/2021 virus belonged to the B1.2. lineage. It was not clustered with other SARS-CoV-2 identified from companion animals in the U.S. (Figure 1).

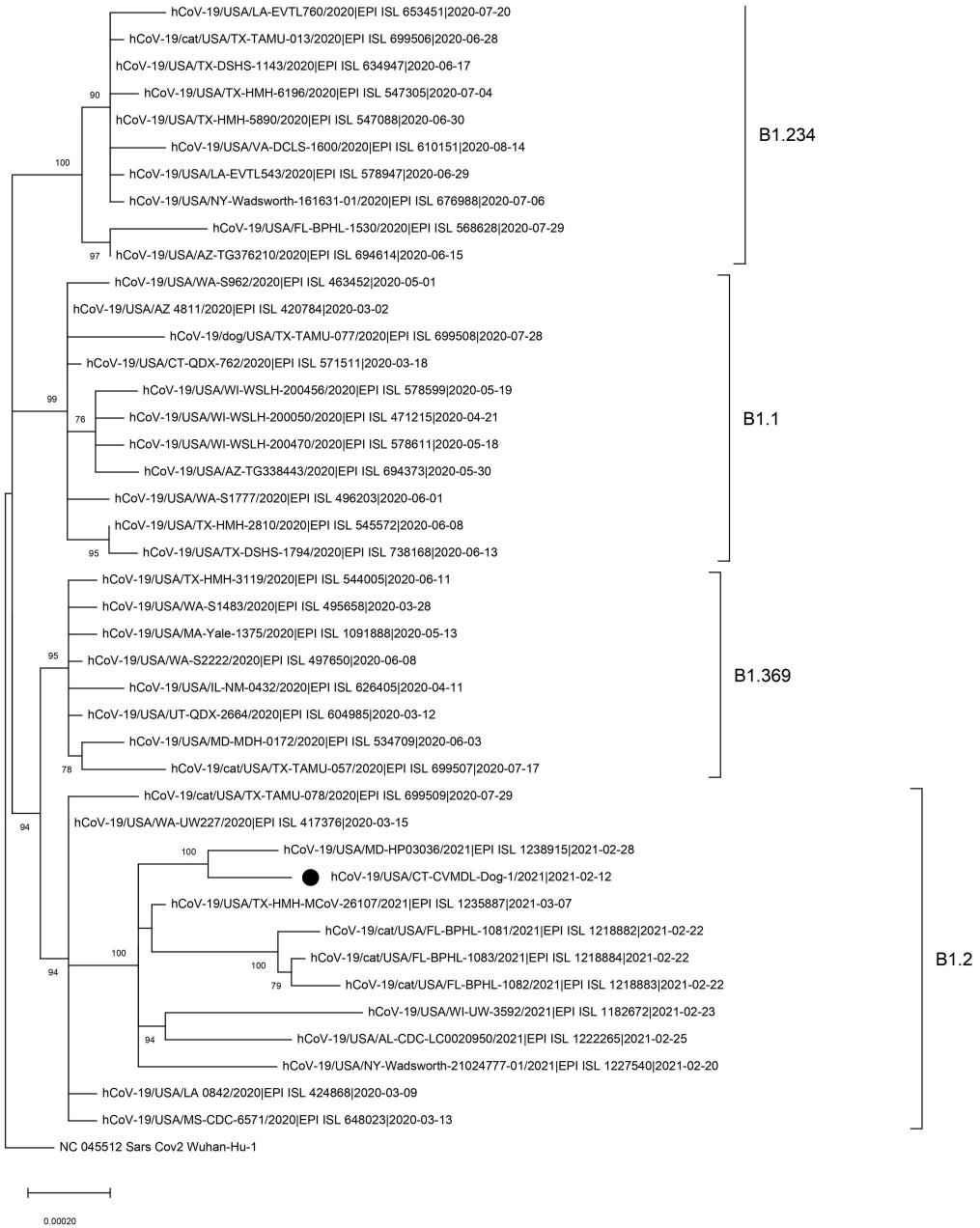

Figure 1. Maximum-likelihood phylogenetic tree of the full-length SARS-CoV-2 genome sequences. The tree was rooted to the Wuhan-Hu-1 virus. Bootstrap values over 70% are shown next to the branches. Scale bar indicates nucleotide substitutions per site. The black circle identifies the hCoV-19/USA/CT-CVMDL-Dog-1/2021 (CT_Dog/2021) virus. The genetic lineages assigned using the PANGOLIN v2.0 (https://github.com/hCoV-2019/pangolin) are indicated with brackets.

We found amino acid substitutions in Spike (D614G), N (D377Y, P67S, P199L), NS3 (G172V, Q57H), NS8 (S24L) NSP2 (T85I), NSP4 (M458I), NSP5 (L89F), NSP12 (P323L), NSP14 (N129D), NSP16 (R216C) proteins. It did not contain any mutations related to the South African variant 'B.1.351' (N501Y, E484K and K417N in Spike) or the U.K. variant "B.1.1.7" (69/70 deletion, N501Y, and P681H in Spike). The B.1 and its sub-lineages that carry both D614G in spike and P323L in nsp12 substitutions have become the dominant variants across the world [9]. The D614G and P323L occurred in China on January 24, 2020 and in the U.K. on February 3, 2020, respectively. Both mutations were first detected

in the U.S. on February 28, 2020 and then has become the dominant mutations in the U.S [10].

The role of companion animals in the evolution and spread of SARS-CoV-2 remains uncertain. Although we did not find direct evidence for transmission between the owner and the dog in this case, it has been reported that SARS-CoV-2 repeatedly spilled over from human to companion animals, highlighting the need for enhanced surveillance in animals. It is of great concern that companion animals would become new reservoir species of SARS-CoV-2 since they are susceptible to infection and could excrete infectious virus.

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Conflicts of Interest: The authors declare no conflict of interest.

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