First genome sequencing of SARS-CoV-2 recovered from an infected cat and its owner in Latin America

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Abstract

An 11 years-old male mixed-breed cat, with exclusively indoor life, presented 3 cough episodes after the owners tested positive by RT-PCR for SARS-CoV-2. The house is inhabited by 5 people (3 adults and 2 children), and 2 of the adults have shown mild symptoms associated with throat discomfort. The cat was vaccinated, had no history of any previous disease, and tested negative for Feline Coronavirus (FeCoV), Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV). Rectal sample collected from the cat was positive for SARS-CoV-2 by RT-PCR. Viral genome sequences recovered from human and cat samples showed an average 99.4% sequence identity. This is the first report of genome sequences of SARS-CoV-2 recovered from a cat and its owner in Latin America.

Keyword

SARS-CoV-2; Next Generation Sequencing; Cat; Brazil

1- Introduction

Coronaviruses belong to the Coronaviridae family, which comprises a group of enveloped single-stranded RNA viruses. The SARS-CoV-2, which is the causal agent of

the new Coronavirus Disease of 2019 (COVID-19), is closely related to bats and pangolins coronaviruses (Zhang, Wu, & Zhang, 2020a; Zhou et al., 2020).

Since the World Health Organization declared the pandemic situation related to COVID-19 (Who, 2020), different animals, including dogs, minks and felines, were reported to test positive for the SARS-CoV-2. In general, the infected animal can become ill, but the clinical manifestation seems to be mild and self-limited (Belgium oie, 2020; Davidson, 2020a; Davidson, 2020b; Rijksoverheid, 2020; Sit, 2020a; Sit, 2020b).

Cats, due to close contact with humans, have received great attention of studies aimed at detecting the SARS-CoV-2 virus in animals, since the first report in Belgium in March 2020 (Belgium oie, 2020). In addition, their Angiotensin-Converting Enzyme 2 (ACE2), used by the virus as receptor and entry route, share high amino acid sequence identity with human ACE2 (Wan, Shang, Graham, Baric, & Li, 2020). Noteworthy, to date, there are no reports of SARS-CoV-2 transmission from cats or dogs to humans.

In fact, since the first report in Belgium, some natural infection cases in cats were reported in Hong Kong (Sit, 2020a), United States of America (Davidson, 2020b) and France (Sailleau et al., 2020). In this study, we report the first case of SARS-CoV-2 infection in a cat in Latin America, and describe genomic sequences obtained from the viruses detected in the cat and its owner.

2- Materials and Methods

An 11 years-old male mixed-breed cat, with exclusively indoor life, presented 3 cough episodes after the owners tested positive by RT-PCR for SARS-CoV-2. The house is inhabited by 5 people (3 adults and 2 children), and 2 of the adults have shown mild symptoms associated with throat discomfort.

The cat was vaccinated, had no history of any previous disease, and tested negative for Feline Coronavirus (FeCoV), Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV).

Nasopharyngeal and rectal samples were collected from the cat to SARS-CoV-2 detection. Laboratory confirmation was performed by RT-PCR through the protocol developed by Charité Hospital, Universitätsmedizin Berlin, Germany (Corman et al., 2020), using GoTaq® Probe 1-Step RT-qPCR System (Promega). This protocol is based on the detection of the SARS-CoV-2 E gene using a pair of primers (F: 5'-3' ACAGGTACGTTAATAGTTAATAGCGT; R: 5'-

3’ATATTGCAGCAGTACGCACACA) and a TaqMan probe (MGB FAM-ACTAGCCATCCTTACTGCGCTTCG) for the detection of the subgenus Sarbecovirus, which includes the causative agent of SARS (SARS-CoV) and the SARS-CoV-2 viruses. As an endogenous control of the reaction, the RNaseP gene was detected using a pair of primers (F: 5’-3’ AGATTTGGACCTGCGAGCG; R: 5’-3’ GAGCGGCTGTCTCCACAAGT) and a TaqMan probe (MGB FAM-TTCTGACCTGAAGGCTCTGCGCG) (Who, 2009). The RT-PCR was carried out on QuantStudio™3 – 96-Well 0.2 mL Block (Thermo Fisher) with holding stage of 45 °C for 15 min and 95 °C for 2 min, followed by 45 cycles of 95 °C for 15 sec and 58 °C for 30 sec.

Total RNA extracted from swab samples was reverse-transcribed with SuperScript™ VILO™ cDNA Synthesis Kit (Invitrogen, Thermo Fisher Scientific, CA, USA) and viral genome amplification and sequencing was performed with the Ion AmpliSeq™ SARS-CoV-2 Research Panel (Thermo Fisher Scientific, Massachusetts, USA), using an Ion 540 chip in the Ion S5 instrument (Thermo Fisher Scientific). Raw sequencing data are available at NCBI SRA database under accession numbers SRR12980649 and SRR12980650.

High-quality reads (Phred> 20) larger than 30 nt were aligned to the NCBI’s SARS-CoV-2 reference genome MN908947.3 using Bowtie2 allowing 1 mismatch (Langmead, & Salzber, 2012). SAM files were processed using SAMtools (Li et al 2009). Then, *de novo* contigs assembly was done using SPAdes (Bankevich et al., 2012) with the parameters “–ion-torrent”, “--mismatch-correction” e “--sc” set following assembler authors’ recommendations for IonTorrent-derived reads. We discarded contigs smaller than 300 nt. For sequence similarity analysis contigs were aligned to the SARS-CoV-2 reference genome using BlastN from BLAST package (Altschul, Gish, Miller, Myers, & Lipman, 1990). The SARS-CoV-2 reference genome, the aligned reads, and the assembled contigs were loaded in IGV browser (Thorvaldsdóttir, Robinson, & Mesirov, 2013) for visual inspection of reads coverage. Consensus of Spike gene was generated mapping reads against reference Spike gene using Bowtie2 and Samtools through *pileup* subprogram. Hierarchical clustering was performed based on percent identity matrix calculated from multiple alignment of Spike gene and plotted as heatmap. Clustering was performed using Pearson correlation with *mcquitty* as grouping method. Multiple alignment was performed using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/).

3- RESULTS AND DISCUSSION

The two owners and the cat were positive for SARS-CoV-2 by RT-PCR, with cycle threshould (Ct) values of 33.836, 35.834, and 36.657, respectively. Since they presented high Ct values, we decided to perform Next Generation Sequencing (NGS) using an Amplicon-based strategy, which is more sensitive for complex samples. Indeed, swab samples of one owner and from the cat were submitted to NGS and both yielded recovery of SARS-CoV-2 genomic sequences with high genome coverage. Viral genomes recovered from human and cat samples showed an average 99.4% sequence identity, as seen in Figure 1A.

This is the first report of genome sequencing of SARS-CoV-2 in a cat from Latin America. The high sequence identity found, suggests that the cat might have been infected by his owner, since it was previously negative for FeCoV, and lives strictly indoors. Indeed, identity-based analysis from multiple alignment using Spike gene indicate that the viruses recovered from cat and human sequenced in this study groups together with SARS-CoV-2 viral genomes identified in other animals, such as those found infecting lion, tiger, cat and dog, and presented low relationship with Feline Coronavirus (Figure 1B). In addition, we also observed that samples sequenced in this study grouped together in the same clade, reinforcing the close relationship among them (Figure 1B). It is worth mentioning that the cat lives in an apartment on the 7th floor and has no contact with any person or any other animal besides the other cat that lives in the house (strictly indoor also), which tested negative for SARS-CoV-2 by RT-PCR.

Regarding clinical presentation, the cat showed mild self-limiting clinical signs, as seen in other reports (Belgium oie, 2020; Davidson, 2020b; Sailleau et al., 2020). In addition, the cat was positive only in the rectal swab, similar to what occurred in the case reported in France (Sailleau et al., 2020), suggesting that this sampling site is important for animals, in addition to being easily accessible for the species in question.

Finally, it is important to notice that there is no proof currently that dogs and cats can transmit COVID-19 to humans, and probably they have no important epidemiological roles on transmission. In a research conducted in New York City, during the pandemic peak in the city, only 3 cats were positive, in a city full of cats (Davidson, 2000a). In Wuhan, about 11% of 102 cats were positive in ELISA after the outbreak (Zhang, Zhang, Gao, Huang, Yang, & Hui, 2020b). Also, there is a low

number of diagnosed animals compared to the numbers of cases in humans, showing that the transmission between humans to cats is not common. Considering our report and previous studies, the authors of the present report do not recommend any kind of abandonment of cats or dogs because of SARS-CoV-2, once these animals do not seem to have any role in transmission or infection in humans.

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ETHICAL STATEMENT

We declare that ethical statement is not applicable.

CONFLICT OF INTEREST

There was no conflict of interest with others.

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Figure 1. Genomic analysis of SARS-CoV-2 genomes isolated from cat or human samples. (A) Contig coverage of samples derived from cat or human. Contigs assembled are shown in red segments. Density of reads covering SARS-CoV-2 reference genome is shown in grey. Percentage of Coronavirus reference genome covered by assembled contigs or reads are shown within parenthesis. Scale of read density is indicated within brackets. Viral sequences from human and cat showed an average 99.4% sequence identity. (B) Hierarchical clustering based on percent identity matrix calculated from multiple alignment of Spike gene recovered from SARS-CoV-2 identified in cat or human samples, other mammals and Feline coronavirus. Sketch of human or cat in the clustering indicate the position of the viral species derived from each organism in the clade. NCBI nucleotide or GISAID database accessions are indicated on the heatmap.