



# SARS-CoV-2 infection in free roaming dogs from the Amazonian jungle

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## ABSTRACT

During the ongoing COVID-19 pandemic, there were several reports of SARS-CoV-2 transmission from human to animals, mostly to companion cats and dogs but also to free ranging wild species like minks and deers. Under this scenario, SARS-CoV-2 surveillance in domestic animals to assess the risk of transmission between species have been suggested by the OIE. Here we present a case report of SARS-CoV-2 infection in free roaming dogs, found at a rural indigenous community from the Ecuadorian Amazonia. Oral and nasal swabs samples were collected from three dogs found during a COVID-19 surveillance intervention in Amazonian indigenous communities where severe COVID-19 outbreaks were suspected. Total RNA was extracted from dog samples and detection of SARS-CoV-2 gene targets N, ORF1ab and S was performed. The three dogs tested positive for at least two SARS-CoV-2 viral targets. Moreover, there was a high SARS-CoV-2 infection rate of 87.2% within this community. Given that 17.1% of SARS-CoV-2 positive individuals had an ultra high load greater than  $10^8$  copies/ml, transmission from humans to dogs likely occurred. To our knowledge, this study is the first report of SARS-CoV-2 positive free roaming dogs. Also, as those animals were found in the Amazonian forest, SARS-CoV-2 transmission to wild mammals is a potential concern. Given the high presence of free roaming dogs associated to rural and indigenous communities in South America, the potential role of these domestic animals on COVID-19 spread would deserve further surveillance studies involving SARS-CoV-2 detection by PCR and molecular epidemiology based on genome sequencing to confirm human to dog transmission.

## 1. Introduction

The novel coronavirus SARS-CoV-2 that appeared in the Chinese province of Hubei in December 2019 lead to the Coronavirus Disease 2019 (COVID-19) outbreak, resulting in the World Health Organization (WHO) declaring a pandemic in March 2020 [1,2]. Up to the end of September 2021, more than 230 million cases and 4.7 million deaths have been reported worldwide (<https://coronavirus.jhu.edu/map.html>). The Americas are one of the most affected regions, with Brazil and USA among the top countries worldwide for number of COVID-19 cases and deaths (<https://coronavirus.jhu.edu/map.html>). In Ecuador, more than 500,000 cases and 32,000 deaths were reported by the end of September 2021 (<https://www.salud.gob.ec/actualizacion-de-casos-de-coronavirus-en-ecuador/>), and several reports have shown active community transmission in rural settings from the Coastal or Amazon region of the country [3–7].

COVID-19 is a viral zoonosis caused by an identified host, although bats and pangolins are the proposed candidates [8,9]. Moreover, the reverse transmission (zoonanthroponosis) of SARS-CoV-2 from humans to several animal species has been reported worldwide [10–13]. Although both *in silico* and experimental studies have revealed that SARS-CoV-2 transmission to dogs is not as great as that for felines [13,14], human to dog and cat infections have been reported worldwide [8–14]. Additionally, most of the current reports for SARS-CoV-2 infection in dogs were done with household ones [8–14] and did not account for free roaming dogs which present a challenge in middle and low income countries due to the high presence of free roaming domestic animals. These animals are not only present in the street of major cities but also at rural settings where they are fed collectively and have free movement within the community [15,16]. To our knowledge, there are no reports of SARS-CoV-2 infected free roaming dogs from South America or any other region in the world.

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Therefore, the aim of this work is to describe the first report SARS-CoV-2 transmission from human to free roaming dogs during a severe COVID-19 outbreak in a rural indigenous community from the Amazonian Region of Ecuador.

## 2. Material and methods

### 2.1. Sample collection

In July 2020, we visited a small indigenous community in the Ecuadorian Amazonia composed of 47 individuals due to an outbreak of COVID-19. The community was isolated and only accessible by plane. Within the community, there were three free roaming dogs that were fed or pets by multiple community members. Oral and nasal swabs in 0.5 ml TRIS-EDTA (pH =8) were collected from the three adult male dogs. The samples were kept in ice until processing in the laboratory from “Universidad de Las Américas” in Quito.

*RNA Extraction and RT-qPCR for SARS-CoV-2 detection using 2019-nCoV CDC kit (IDT, USA), “TaqMan 2019-nCoV Assay Kit v1” (ThermoFisher, USA) and 2019-nCoV kit (Da An Gene, China).* The three samples from dogs were tested following an adapted version of the CDC protocol: [1] using AccuPower Viral RNA extraction kit (Bioneer, South Korea) as an alternate RNA extraction method; [2] using CFX96 BioRad instrument [17–22]. RT-qPCR for SARS-CoV-2 detection was performed with 3 different commercial kits following each manufacturer's instructions: “2019-nCoV CDC kit” (IDT, USA) that includes viral targets N1 and N2; “2019-nCoV kit” (Da An Gene, China) that includes viral targets N and ORF1ab; “TaqMan 2019-nCoV Assay Kit v1” (ThermoFisher, USA) that includes the viral targets N, S and ORF1ab. The three dog samples were sent to Macrogen (South Korea) for whole genome sequencing.

### 2.2. Ethics statement

The human data included in this work is part of a retrospective COVID-19 epidemiology project approved by the Institutional Review Board from Hospital General San Francisco (Quito) with code CEISH-HGSF-2021-002. The animal sample collection and analysis was approved by UDLA “Comité de Ética y Bienestar Animal”, following the Ecuadorian regulations for animal research (“Ley Orgánica de Sanidad Agropecuaria” 2017, Asamblea Nacional, República del Ecuador”).

## 3. Results

The three dogs have no clinical signs of COVID-19 but tested positive for at least two SARS-CoV-2 gene targets N1 and N2 by RT-qPCR; but also, two of the dogs were also positive for ORF1ab gene target, and one of the them for S gene target. Ct values ranged from 34.8 (N1 target with 2019-nCoV CDC kit for sample 1634) to 41.7 (ORF1ab with 2019-nCoV kit from Da An Gene for sample 1634) See [Table 1](#) for detailed Ct values for all the gene targets for the three dogs. Overall, all the three dogs tested positive for N1 and N2 viral gene targets with 2019-nCoV CDC kit, fulfilling criteria for human positive cases established by the Center for Disease Control and Prevention (USA). Moreover, the dog samples 1633

**Table 1**

Ct values for the different SARS-CoV-2 gene targets for the three dogs included in this study. Three different commercial RT-qPCR kits for SARS-CoV-2 detection were used (NA means “not amplified”).

Dog sample code	2019-nCoV CDC (IDT, USA)		2019-nCoV kit (Da An Gene, China)		TaqMan 2019-nCoV Kit v1 (ThermoFisher, USA)	
	N1	N2	N	ORF1ab	S	ORF1ab
	D-1633	36.9	38.9	40.79	NA	NA
D-1634	34.8	36.7	39.92	41.7	38.48	39.92
D-1635	35.4	35.5	NA	NA	NA	NA

and 1634 were also positive for N viral gene target with 2019-nCoV kit and for ORF1ab viral gene target with TaqMan 2019-nCoV Assay Kit v1. The dog sample 1633 was also positive for viral gene target S with TaqMan 2019-nCoV Assay Kit v1. Additionally, the dog D1633 tested negative for ORF1ab viral gene target with 2019-nCoV kit and for S viral gene target with TaqMan 2019-nCoV Assay Kit v1; and the dog D1635 tested negative for ORF1ab viral gene target with 2019-nCoV kit and for S and ORF1ab viral gene targets with TaqMan 2019-nCoV Assay Kit v1.

The three dog samples sent to Macrogen (South Korea) for whole genome sequencing did not yield reliable sequences alignment reflecting the high Ct values.

Regarding the individuals in the community, we could access retrospectively to the anonymized data for SARS-CoV-2 RT-qPCR detection. From the 47 people tested, 41 were positive yielding an infection rate of 87.2%. Seven individuals among the positive ones carried a SARS-CoV-2 viral load bigger than  $10^8$  copies/mL.

## 4. Discussion

This is the first report of SARS-CoV-2 positive dogs in South America and to the best of our knowledge the first report in free roaming dogs. Other surveillance studies involving cats and dogs in Latin America (Chile and Mexico), focused on companion animals and also only SARS-CoV-2 positive cats were found [23,24].

Our dog samples collection was carried out while an active SARS-CoV-2 surveillance program was conducted on Amazonian indigenous communities from Ecuador. As part of the surveillance activity, we tested three free roaming dogs that had close community contact. Those dogs did not have any respiratory symptoms by the time that samples were collected, they were free ranging dogs within the community, and were pet and fed collectively by the community members. Moreover, these dogs are also in close contact with humans and other wild animals are they are involved in the daily hunting and food gathering activities of this indigenous community.

Seven individuals had viral loads that have been associated to super spreading events [25] which would have included possible spread to the free roaming dogs which had close community contact. Moreover, dog to dog transmission could not be neither rule out. The low viral loads found in the dogs did not allow SARS-CoV-2 whole genome sequencing. In fact, these low viral loads would explain why some of the SARS-CoV-2 gene targets failed to test positive in two of the dogs as it is detailed in the results section as the differences in sensitivity for the several gene target included in this study may yield false negative results for Ct values in the range of 35 to 40.

Although the potential role of domestic dogs in COVID-19 spread has been considered unlikely [26–28], those conclusions arise from studies developed on high income countries where fewer free roaming dogs are found that at middle and low income countries. As we are showing in this report, further research is needed regarding the role of companion animals in SARS-CoV-2 transmission them to other companion and wild animals or even to humans in the context of low and middle income countries were the occurrence of free roaming and feral dogs and cats, either in rural or urban areas, is frequent [15,16].

It is important to notice that other surveillance studies for companion animals from COVID-19 positive owners did not report SARS-CoV-2 detection by RT-qPCR in dogs [26–28]. In these studies, the samples were collected more than ten days since the owner tested positive for SARS-CoV-2 infection [26–28]. However, in our study, the dog samples were collected while the COVID-19 outbreak was happening in the community. Differences in surveillance study detection, either serology or RT-qPCR, in domestic animals could reflect that the infective period between the human diagnosis and animal diagnosis is not known [8–12,26–28]. Nevertheless, although not surveillance studies, there are two reports involving necropsies of dogs and cats diagnosed with COVID-19 by RT-qPCR [29,30].

Additionally, the geographical location of the free roaming dogs

included in the current study represent a potential risk in terms of public health and conservation. There are more than 300 mammal species in Ecuador, including several vulnerable, endangered or critically endangered ones according to the International Union for Conservation of Nature (IUCN) red list of threatened species [31], most of them present at the Ecuadorian Amazonian. SARS-CoV-2 infection has already been described in the wild animals like minks and white tailed deer [32,33], showing that anthropogenic SARS-CoV-2 transmission has already created new viral uncontrolled reservoirs or unknown effects in the wild mammals health. Moreover, as SARS-CoV-2 infection has been described in captive primates and felines [8], we particularly point out the potential risk for the critically endangered primate Ecuadorian capuchin (*Cebus aequatorialis*); and the felines species of oncilla (*Leopardus tigrinus*) and jaguar (*Panthera onca*), vulnerable and near threatened respectively, according to the IUCN list [31]. All those three species are present within the area where free roaming SARS-CoV-2 infecting dogs were found and could be potentially affected for a dog to wild animals SARS-CoV-2 transmission that may risk their survival [34].

The main limitation in our study is the lack of genomic information regarding SARS-CoV-2 lineages infecting human and dogs. Although we tried to assess WGS from dog samples, the low viral loads obtained for the three SARS-CoV-2 positive dogs did not allow to obtain WGS good quality data. Additionally, there are strong bioethical regulations protecting the genomic information for indigenous ethnic groups in Ecuador, and our IRB approval did not allow to use the collected samples for any other aim than SARS-CoV-2 detection by RT-qPCR. Nevertheless, considering the high infection rate of almost 90% found within the community, the presence of individuals with high viral loads and that all the dogs tested positive for two or more SARS-CoV-2 gene target, our results support that anthropogenic SARS-CoV-2 transmission is the most plausible explanation for the infection of the free roaming dogs included in this study.

#### Authors' contributions

- All authors contributed towards sample collection and data analysis.
- MAGB composed the manuscript.
- All authors read and approved the final manuscript.

#### Declaration of Competing Interest

None.

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