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# SARS-CoV-2 transmission from infected owner to household dogs and cats is associated with food sharing



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## ABSTRACT

**Objectives:** Several cases of reverse transmission of SARS-CoV-2 from human to pets were reported during the first year of the COVID-19 pandemic. Accordingly, the World Organization for Animal Health has recommended to improve SARS-CoV-2 surveillance on household animals to assess the risk of transmission between species. After such recommendation, we studied the potential SARS-CoV-2 infection in household dogs and cats in the city of Guayaquil, the most populated city in Ecuador.

**Methods:** Oral and nasal swab samples were collected from dogs and cats within 10 days of a positive SARS-CoV-2 test result of their owners. Total ribonucleic acid was extracted and detection of viral gene targets N and ORF1ab was performed by quantitative reverse transcription polymerase chain reaction.

**Results:** From the 50 cats and dogs tested, 12 were SARS-CoV-2 positive, giving a total positivity rate of 24%. A total of 1 of 8 cats tested positive, whereas 11 of 42 dogs were positive, yielding a positivity rate of 12.5% and 26.2%, respectively. SARS-CoV-2 was confirmed by whole genome sequencing. In addition, we also found a statistically significant association between SARS-CoV-2 pet positivity and food sharing with infected owners.

**Conclusion:** This study is the second active surveillance of SARS-CoV-2 in household dogs and cats in Latin America. Moreover, it is the first study to address the risk factors associated with potential anthropogenic SARS-CoV-2 transmission to domestic cats and dogs. Given the high presence of free-roaming dogs and cats in rural and urban areas in Latin American countries and the high capacity shown by coronaviruses for interspecies transmission, our findings support the view that SARS-CoV-2 surveillance in pets is necessary to better understand the role that pet-human interaction plays in the COVID-19 spread.

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## Introduction

The rise of SARS-CoV-2 in the Chinese province of Hubei in December 2019 led to the SARS-CoV-2 outbreak that prompted the World Health Organization to declare a pandemic on March

11, 2020 (Gorbalenya et al., 2020; Zhou et al., 2020; Ortiz-Prado et al., 2020). By the end of March 2022, more than 470 million cases and 6 million deaths have been reported worldwide (<https://coronavirus.jhu.edu/map.html>). The Americas is one of the most affected regions, with millions of cases and deaths. In Ecuador, more than 700,000 cases and 32,000 deaths were reported by March 2022 (<https://www.salud.gob.ec/actualizacion-de-casos-de-coronavirus-en-ecuador/>).

SARS-CoV-2 is a zoonotic virus whose origins and intermediate hosts have been associated with bats and pangolins (Tiwari et al.,

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2020). Furthermore, few months after the COVID-19 pandemic outbreak, the reverse transmission of SARS-CoV-2 from humans to domestic and wild animals have been reported worldwide (OIE, 2020; Hobbs et al., 2020; Mahdy et al., 2019). According to the World Organization for Animal Health (OIE), more than 20 countries have reported natural infections of SARS-CoV-2 in domestic animals (OIE, 2020). In this scenario, the OIE recommends implementing active epidemiologic surveillance and report of SARS-CoV-2 infections in other animal species, especially for those in close contact with humans, such as pets (OIE, 2020; Hobbs et al., 2020). Although both *in silico* and experimental studies have revealed that SARS-CoV-2 transmission could be less frequent in canines than in felines (Martínez-Hernández et al., 2020; Shi et al., 2020), naturally-infected household cats and dogs have been reported worldwide, with similar prevalence values, ranging from 0–17.65% for cats and 0–13.33% for dogs (OIE, 2020; Hobbs et al., 2020; Shi et al., 2020; Barua et al., 2021; Sánchez-Montes et al., 2021; Mahdy et al., 2019). Although most of these cases are asymptomatic, the presence of respiratory symptoms has been previously described (Garigliany et al., 2021; Barrs et al., 2020).

Most of the current reports of SARS-CoV-2 infection in household cats and dogs come from high-income countries (OIE, 2020; Hobbs et al., 2020; Mahdy et al., 2019). However, as free-roaming dogs are more frequent in middle- and low-income countries, the potential role of pets on SARS-CoV-2 transmission may represent a major public health threat (Orlando et al., 2019; Charlotte et al., 2021). For instance, the free-roaming dog population in Guayaquil (the most populated city of Ecuador), is greater than 30,000 (Orlando et al., 2019). However, SARS-CoV-2 surveillance studies in cats and dogs in low- and middle-income countries like those in Latin America are still scarce, and only a few reports from Chile, Argentina, and Mexico have been published to date (Neira et al., 2021; Pecora et al., 2022).

The aim of this work was to study the SARS-CoV-2 infection rate in household cats and dogs owned by patients with COVID-19 in the city of Guayaquil, in the coastal region of Ecuador, where COVID-19 community transmission has been present since the initial outbreak in February 2020.

## Material and methods

### Study design

A cross-sectional surveillance study was carried out in the city of Guayaquil, a province of Guayas in the coastal region of Ecuador, which included 50 pets (42 dogs and 8 cats). A prospective sampling was carried out in cats and dogs owned by individuals who were diagnosed with COVID-19 and confirmed by quantitative reverse transcription polymerase chain reaction (RT-qPCR) testing within 10 days before pet sample collection. In addition, a risk factor survey was performed for each household included in the study. The sample collection was carried out during the months of March to May 2021. Households with one or more pets were included in the study and all the pets in each household were tested.

The variables included for the risk factors survey were sex and species of pet, and the following binary variables with yes or no as answers were included: bed sharing between pet and owner, food sharing with pet, pet COVID-19-related symptoms, pet access to trashed food, isolation of the pet from the owner after COVID-19 diagnosis, presence of other animals in the household, and pet-free access to the street.

### Sample collection and RNA extraction

Household animals were physically restrained for nasal and oral swab sample collection in 1 ml of saline. The samples were pre-

served in a cold chain until processing in Instituto Nacional de Investigación y Salud Pública laboratory in Guayaquil (Ecuador). The viral ribonucleic acid (RNA) extraction was carried out using a column-based commercial kit from Biomiga Inc. (San Diego, California, USA), following manufacturer's protocol and as has been previously done in our laboratory (Freire-Paspuel et al., 2020; Freire-Paspuel et al., 2021; Santander-Gordon et al., 2021).

### SARS-CoV-2 detection by RT-qPCR

RNA extracted samples were subjected to RT-qPCR for SARS-CoV-2 detection using the United States Food and Drug Administration emergency use-authorized certified commercial kits, 2019-nCoV Nucleic Acid Diagnostic Kit (Sansure Biotech, China), including two viral gene targets: N and ORF1ab. The RT-qPCR reactions were performed, following the manufacturer's instructions. For RNA quality control, human RNAaseP amplification was considered valid as a proxy for extraction quality control, and spectrophotometric measurement of RNA extraction quality was also done.

### Whole genome sequencing

Four of the SARS-CoV-2 RT-qPCR positive samples were submitted to whole-genome sequencing in a MinION device (Oxford Nanopore Technologies), following the ARTIC V2 protocol (Artic Network, 2021). The genomes were processed using the Artic bioinformatics pipeline and the reads aligned using the Burrows-Wheeler Aligner tool (Li, 2010). Genomes annotated with this tool were downloaded and aligned in BioEdit (Hall and Carlsbad, 2021) with SARS-CoV-2 as the reference genome sequenced early in the pandemic in Wuhan, "isolate Wuhan-Hu-1" GenBank ID: NC\_045512.3. Genome alignment using CLUSTAL-W and phylogenetic analysis using maximum likelihood method with a GTR substitution model and 1000 bootstrap iterations was conducted using IQ-tree 2.2.0 (Minh et al., 2020) to attempt identifying the SARS-CoV-2 lineages infecting dogs that tested positive.

### Statistical analysis

Inferential analysis was performed using RStudio statistical software. Significant difference between the presence of SARS-CoV-2 and risk factors were determined using chi-square analysis, whereas significant factors in the presence of SARS-CoV-2 were determined using a logistic regression model.

## Results

A total of 50 samples from household animals were collected at the city of Guayaquil, including 42 samples from dogs and 8 samples from cats. Regarding sex, 24 samples were collected from male pets and 26 from female pets. The ages of the sampled animals ranged from 4 months to 16 years. Eleven samples from dogs (11/42 = 26.2%) and one sample from cats (1/8 = 12.5%) were positive for N1 and/or ORF1ab SARS-CoV-2 viral genes, with cycle threshold (Ct) values ranging from 30.11–39.36 (see Table 1). Overall, 12 pets of the 50 tested were positive for SARS-CoV-2 as assessed by RT-qPCR, yielding an overall SARS-CoV-2 infection rate of 24%.

The bivariate and multivariate analysis for the risk factor included in our survey is detailed in Table 2. For the bivariate analysis, male pets were statistically associated with a higher risk of infection ( $P = 0.027$ ). For the multivariate analysis, both being male and sharing food with the owner were statistically associated with SARS-CoV-2 infection, with a regression coefficient of 0.26 and  $P$ -values of 0.029 and 0.025, respectively.

**Table 1**

Ct values for the viral gene targets for pets who were SARS-CoV-2 RT-qPCR-positive included in the study.

	Sample code	Species	Sex:	Ct values	
				N viral target	ORF1ab viral target
1	DF8	dog	F	30,11	32,04
2	DF10	dog	M	35,47	39,36
3	DF12	dog	F	37,21	36,52
4	DF17	dog	M	34,30	36,25
5	DF26	cat	F	36,53	NA
6	DF33	dog	M	37,07	NA
7	DF37	dog	M	33,57	38,11
8	DF39	dog	M	37,26	NA
9	DF41	dog	M	32,46	35,10
10	DF42	dog	M	37,95	NA
11	DF44	dog	F	30,86	33,51
12	DF45	dog	M	31,76	35,08

Ct: cycle threshold; F: female; M: male; NA: not amplified.

Whole genome sequencing from four positive samples confirmed SARS-CoV-2 infection in the pets. Three sequences have enough quality and were submitted to GenBank: accession numbers MZ322504, MZ322502, and MZ322409. The genome coverage obtained ranged between 45% and 85%. Sequences were aligned and visualized in BioEdit, showing 98–100% similarity to the viral reference genome isolated in Wuhan early in the pandemic. Alignment analysis conducted with the online tool Basic Local Alignment Search Tool yielded a SARS-CoV-2 identity up to 99.58%. The phylogenetic analysis with the sequences included, clustered all sequences with the early lineages of SARS-CoV-2 circulating in Ecuador during 2020–2021: two into the cluster containing lineage 19 A and 19 B and one with lineage Iota (Figure 1).

## Discussion

This study is the second active surveillance of SARS-CoV-2 infection in populations of household dogs and cats in Latin America, and the first one to report dogs who were SARS-CoV-2 positive. The other surveillance study involving cats and dogs in Latin America was carried out in Mexico, and no infected animals were found (Sanchez-Montes et al., 2021). In addition, two case reports of transmission from human to cats in households in Chile and Argentina have recently been published (Neira et al., 2021; Pecora et al., 2022).

A SARS-CoV-2 infection rate of 24% was found by RT-qPCR. Moreover, the infection rate was higher in dogs (26.2%) than in cats (12.5%), although this result should be taken with caution because potential bias due to the small sample size for cats cannot be ruled out. In our study, the samples from pets were collected within 10 days of the pet's owner positive RT-qPCR test for SARS-CoV-2. Previous reports in household cats have shown RT-qPCR SARS-CoV-2 detection in nasal and rectal samples that were collected within 2 weeks of their owners' COVID-19 diagnosis (Barrs et al., 2020; Garigliany et al., 2020). Two additional surveillance studies including cats and dogs in Italy and Spain detected no RT-qPCR SARS-CoV-2-positive results in dogs and only a single positive result in cats (Patterson et al., 2020; Ruiz-Arrondo et al., 2021). However, in these studies, the time window used to collect samples after the owners' positive COVID-19 result was longer. Different time frames between the pet owners COVID-19 diagnosis and the pet sample collection could explain the discrepancy among the available reports. To date, the effective time window for SARS-CoV-2 detection by RT-qPCR in pets have not been assessed, and negative results should be taken with caution.

Finally, those reports in which SARS-CoV-2 has been detected in pets show that close contact with an infected owner poses a risk to pets, although no risk factor analysis has been carried out yet (Barrs et al., 2020; Garigliany et al., 2020; Patterson et al., 2020; Ruiz-Arrondo et al., 2021). To the best of our knowledge, our study is the first to address a risk factor analysis for SARS-CoV-2 transmission from human to domestic cats and dogs. We found that food sharing between infected owners and their pets was associated with SARS-CoV-2 infection. A limitation in our study was that this risk factor association was based on a survey done retrospectively and no samples from food were taken at the time of pet sample collection. Nevertheless, this result would suggest a potential important role of fomites for human-to-pet SARS-CoV-2 transmission in addition to human-to-human transmission that has been mainly associated with aerosols (Onakpoya et al., 2021).

A strong limitation in our study is lack of information regarding SARS-CoV-2 variants infecting cat and dog owners. Our phylogenetic results also need to be interpreted with caution because phylogenies depend on the quality of sequences and the Ct values found in dogs were not optimal for getting better genome coverage, particularly of the Spike gen. Taking this into account, we inferred two dogs that tested positive were infected with early lineages of SARS-CoV-2 and one dog with the Iota vari-

**Table 2**

Bi- and multivariate analysis of association of risk factor in relation to SARS-CoV-2 infection in pets (42 dogs and 8 cats) included in the study.

Risk factor		SARS-CoV-2 (+)	SARS-CoV-2 (-)	Bivariate analysis		Multivariate analysis	
				OR (IC 95%)	P-value	OR (IC 95%)	P-value
Specie	Dog	11	31	1.92 (0.06–60.59)	0.71	2.07 (0.09–46.8)	0.79
	Cat	1	7				
Sex	Female	4	22	14.46 (0.92–228.2)	0.027 <sup>a</sup>	14.46 (0.92–228.2)	0.029 <sup>a</sup>
	Male	8	16				
Bed sharing with owner	Yes	9	21	2.02 (0.23–17.81)	0.52	2.3(0.27–19.6)	0.37
	No	3	17				
Food sharing with owner	Yes	8	20	2.62 (0.04–151.9)	0.61	6.17 (0.22–167.4)	0.025 <sup>a</sup>
	No	4	18				
Symptoms	Yes	4	10	2.19 (0.18–27.1)	0.54	0.89(0.10–7.4)	0.91
	No	8	28				
Trash access	Yes	3	10	7.33 (0.13–428.5)	0.33	2.08(0.06–69.2)	0.84
	No	9	28				
Pet isolation	Yes	11	34	1.22 (0.13–12.83)	0.32	0.44(0.00–13.7)	0.82
	No	1	4				
Other pets	Yes	7	29	0.22 (0.01–9.51)	0.43	0.07(0.00–1.9)	0.08
	No	5	9				
Free street access	Yes	1	8	0.05 (0–6.65)	0.21	0.13(0.00–8.4)	0.61
	No	11	30				

<sup>a</sup> statistically significant association. IC: confidence interval; OR: odds ratio.



**Figure 1.** Phylogenetic placement of SARS-CoV-2 sequences obtained from RT-qPCR positive dogs in Guayaquil (Ecuador) in comparison with selected genomes of different viral lineages detected in human samples in Ecuador from 2020–2021. The tree was obtained using maximum likelihood method with a GTR substitution model and 1000 bootstrap iterations was conducted using IQ-tree 2.2.0. Dog samples are presented in red with GenBank accession number. Lineage name and GISAID accession number is presented for all other sequences. GTR: general time reversible; RT-qPCR: quantitative reverse transcription polymerase chain reaction.

ant. Sit et al. (2020) showed that early SARS-CoV-2 lineages are able to infect dogs in a study conducted in Hong Kong, whereas Miro et al. (2021) demonstrated a dog's infection with the Alpha variant in a survey in Spain. Unfortunately, we did not have the institutional review board approval to obtain SARS-CoV-2 samples from pet owners by the time this study was done, although a positive RT-qPCR result for the owner done at a Ministry of Health-certified laboratory was mandatory as part of the inclusion criteria. Nevertheless, the pets included in this surveillance were permanently staying within the household during the time of the owners' isolation due to COVID-19 diagnosis and also close exposure to owners due to food sharing was a risk factor. Therefore, although we cannot totally rule out other sources of infection for the SARS-CoV-2-positive pets because no sequences from the owners were available, we believe that anthropogenic infection of the pets is the most plausible explanation for our results.

Overall, our results support the need of SARS-CoV-2 surveillance studies in companion animals. Also further research with One Health approach is needed to understand SARS-CoV-2 transmission dynamics in pets in different socioeconomic scenarios, such as in middle- and low-income countries, where household crowding is usually bigger than in high-income countries, and practices like sharing human food leftovers with pets may be more usual.

### Declaration of Competing Interest

The authors have no competing interests to declare.

### Authors' contributions

- All authors contributed toward sample collection and data analysis.

- MAGB and AO composed the manuscript.
- All authors read and approved the final manuscript.

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### Ethical approval

This study was approved by the Scientific Board of the Instituto Nacional de Salud Pública en Investigación. Nevertheless, according to Ecuadorian regulations, infectious disease diagnosis and surveillance in domestic animals do not require institutional review board approval. All the pet owners signed an informed consent.

### Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.ijid.2022.05.049](https://doi.org/10.1016/j.ijid.2022.05.049).



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