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SHORT COMMUNICATION

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Detection of SARS-CoV-2 in pets living with COVID-19 owners diagnosed during the COVID-19 lockdown in Spain: A case of an asymptomatic cat with SARS-CoV-2 in Europe

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Abstract

Pets from COVID-19 owners were screened for SARS-CoV-2 (April–May 2020). From 23 pets, an asymptomatic cat showed positive RT-qPCRs results from oropharyngeal swab (negative rectal swab). Remaining pets were negative. This suggests that cats can contract the virus from their infected owners and may act as potential hosts for SARS-CoV-2. Their role in carrying live or infectious viruses and disseminating them needs more investigation.

KEYWORDS
animals, cat, COVID-19, pets, SARS-CoV-2, Spain

1 | INTRODUCTION

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for the current coronavirus disease 2019 (COVID-19) pandemic, is the seventh coronaviruses that cause human respiratory infections. It belongs to the genus *Betacoronavirus*, such as the two other zoonotic coronaviruses (SARS-1 and MERS) that recently caused outbreaks. The natural origin of SARS-CoV-2 seems derived from bats (spillover). Pangolins, snakes, turtles, hamsters or yaks have been considered as potential intermediate hosts before spreading to humans, but this still remains unclear (Luan, Jin, Lu, & Zhang, 2020). According to World Health Organization (WHO) (May 8, 2020), there is no evidence that dogs and/or cats can disseminate SARS-CoV-2 and act as source of human infection (https://www.who.int/es/emergencies/diseases/novel-coronavirus-2019/advice-for-public/q-a-coronaviruses). Nevertheless, in any new or emerging disease, especially if there are 'knowledge gaps' in the epidemiology, to assess the potential for domestic transmission through pets (mainly, dogs and cats) is relevant (https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/COV-19/COVID19_21Feb.pd f). Our aim was to evaluate, at an early stage of COVID-19 pandemic, the state of infection and, in consequence, the potential role of SARS-CoV-2 transmission from humans to companion animals (especially dogs and cats) under a one health approach in La Rioja (northern Spain).

2 | MATERIALS AND METHODS

A total of 23 asymptomatic mammalian pets under quarantine (eight cats, one guinea pig, two rabbits and 12 dogs) from 17 households with confirmed human cases of COVID-19 infection diagnosed at the Hospital Universitario San Pedro (Logroño, Spain) were included in our study (8 April–4 May 2020) (Table 1). All residences were located in La Rioja (northern Spain). Institutional review board approval was obtained from the Ethical Committee of Research with medicines from La Rioja (CEImLAR), Ref. P.I. 419. Two samples per animal (oropharyngeal and rectal swabs) were collected and preserved at 4°C in 2 ml Dulbecco's modified Eagle medium (DMEM) with penicillin (100 units/mL) and streptomycin (100 μg/ml) (Sigma-Aldrich) up to the arrival (<5 hr) to the Center of Rickettsiosis and Arthropod-Borne Diseases–Center of Biomedical Research from La Rioja (CRETAV-CIBIR). Samples were aliquoted in a class II biological safety cabinet at a BSL-2 facility. Carrier RNA (1 μg) (Qiagen) was added to each 200 μl sample used for RNA extraction with RNeasy Mini Kit (Qiagen). RNA extracts were eluted in 65 μl of RNase-free water and

TABLE 1 Characteristics of pets screened for SARS-CoV-2 living with owners diagnosed with COVID-19 during the quarantine (April–May 2020), in La Rioja (Spain)

Sample ID	Pet	Breed	Age (years)	Gender	SARS-CoV-2 RT-qPCR results		Disease severity of COVID-19 in pet owners	Days from owner's COVID-19 diagnosis to pet sample collection
					Oropharyngeal swab	Rectal swab		
Covid1	Guinea pig	Indeterminate	<1	Male	Negative	Negative	Moderate	6
Covid2	Cat	European	11	Male	Negative	Negative	Moderate	9
Covid3	Cat	European	9	Male	Negative	Negative		
Covid4	Dog	Spanish mastiff	<1	Male	Negative	Negative	Moderate	11
Covid5	Cat	European	6	Male	Negative	Negative	Mild	9
Covid6	Dog	Bichon maltese	11	Female	Negative	Negative	Severe	10
Covid7	Dog	Bichon maltese	11	Male	Negative	Negative		
Covid8	Cat	European	8	Female	Positive	Negative	Severe	4
Covid9	Cat	European	7	Male	Negative	Negative		
Covid10	Dog	Yorkshire	3	Female	Negative	Negative	Moderate	3
Covid11	Dog	Mixed mastiff	10	Male	Negative	Negative	Moderate	3
Covid12	Cat	European	15	Male	Negative	Negative	Moderate	3
Covid13	Dog	Gos d'atura	5	Male	Negative	Negative	Moderate	20
Covid14	Rabbit	Super toy	2	Male	Negative	Negative	Mild	32
Covid15	Rabbit	Teddy	1	Male	Negative	Negative		
Covid16	Dog	Labrador retriever	4	Female	Negative	Negative	Mild	17
Covid17	Dog	Mixed breed	6	Female	Negative	Negative	Mild[a]	29
							Moderate[a]	26
Covid18	Cat	Toyger	6	Male	Negative	Negative	Mild	18
Covid19	Cat	European	4	Female	Negative	Negative		
Covid20	Dog	Mixed Villano–Alano	<1	Female	Negative	Negative	Mild	16
Covid21	Dog	Mixed breed	9	Female	Negative	Negative	Mild	41
Covid22	Dog	Great dane	11	Female	Negative	Negative		
Covid23	Dog	Spanish water dog	3	Female	Negative	Negative	Mild	26

[a]Two confirmed human cases in the same household. Mild: upperway respiratory clinic without radiological data from pulmonary involvement; Moderate: respiratory involvement with radiological alteration without the need for oxygen; and Severe: respiratory involvement with radiological alteration and need for oxygen.

stored at −80°C. DNA was digested using RNase-Free DNase Set (Qiagen). All samples were screened for SARS-CoV-2 using a specific one-step RT-qPCR assay targeting a fragment gene encoding the nucleocapsid (N) of the virus (2019-nCoV_N1 primers and probe set) (https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html) with the One-Step PrimeScript™ RT-PCR Kit (Takara Bio Inc.). A 20 µl reaction was set up containing 5 µl of RNA extract, with 500 nM each primer and 125 nM probe. Thermal cycler was performed at 42°C for 5 min for reverse transcription, followed by 95°C for 10 s and 45 cycles of 95°C for 5 s and 55°C for 30 s. All specimens were also analysed using the commercial kit GPS™ CoVID-19 dtec-RT-qPCR Test (Genetic PCR Solutions™). Positive cases were confirmed by RT-qPCR for the envelope (E) protein-encoding gene (Corman et al., 2020), as described above for the N1 gene, with these modifications: final concentrations of each primer and probe were 400 and 200 nM, respectively, and the annealing took place at 58°C for 30 s. Synthetic plasmid controls with the complete SARS-CoV-2 N gene (Integrated DNA Technologies) and the E gene (Eurofins Genomics) were used to generate standard curves based on tenfold serial dilutions for quantification. The GPS™ CoVID-19 dtec-RT-qPCR Test kit also included a positive control. Positive and negative (extraction and amplification) controls were included in all the RT-qPCR assays. Samples and controls were tested in triplicate.

3 | RESULTS AND DISCUSSION

The oropharyngeal swab sample from a female cat tested positive for SARS-CoV-2 by the three RT-qPCR assays performed (Table 1). The specimen showed a viral load of 1.7×10^3 RNA copies/µL for the N1 fragment gene and 1.1×10^3 RNA copies/µL for the E gene. Viral RNA was not detected in the rectal swab sample from this animal. It was an 8-year-old female domestic European cat without clinical signs related to coronavirus disease, although it had chronic feline gingivostomatitis, feline idiopathic cystitis (treated with glucosamine and chondroitin sulphate), chronic kidney disease (treated with special feeding, ranitidine and benazepril hydrochloride) and feline asthmatic bronchitis (treated with fluticasone propionate). It cannot be ruled out that the virus was found in the swab just because the cat came into contact with a surface/fomites heavily contaminated by the infected owner (e.g. due to the owner's sneezing or coughing), and ingested the virus by licking a contaminated surface or its own body. Nevertheless, animal models have demonstrated that cats are susceptible to infection by SARS-CoV-2, and we think that our finding (viral RNA detection) is indicative of an infection. The cat lived in a two-cat household with a 7-year-old male European domestic cat that tested negative for SARS-CoV-2. The efficient replication and/or transmission of the virus in cats has been experimentally demonstrated with viral loads higher than those detected herein (Halfmann et al., 2020; Shi et al., 2020), but without culture assays we cannot be sure that the virus actively replicated in the positive cat. Follow-up nasopharyngeal and rectal swab samples were subsequently taken on 4 May 2020 (after 26 days), and both specimens from the two cats tested negative. The owner suffered from severe pneumonia, and he was hospitalized for 8 days. Swab samples from the remaining mammalian pets were negative.

Our study reports the detection of an asymptomatic cat with SARS-CoV-2 in Europe, probably associated with close contact with its owner who was diagnosed with active COVID-19 infection. According to our data, a high prevalence of SARS-CoV-2 RT-qPCR-positive cats was observed (1/8; 12.5%). Up to our knowledge, the known prevalence data for cats are those from Zhang et al. (Zhang et al., 2020). They found a 14.7% seroprevalence against SARS-CoV-2 in cats in China. To our knowledge, no previous SARS-CoV2 RNA detection in asymptomatic cats has been reported in Spain. Another case of SARS-CoV-2 infection in a cat without signs of respiratory disease has been reported in Germany (https://www.oie.int/en/scientific-expertise/specific-information-and-recommendations/questions-and-answers-on-2019novel-coronavirus/events-in-animals/). Three cats remained asymptomatic after cohousing and SARS-CoV-2 transmission by infected cats (Halfmann et al., 2020). The remaining SARS-CoV-2 infections in felines (Belgium, USA, France and Spain) were symptomatic and mainly showed clinical signs of respiratory and/or digestive disease. A more recent case has been detected in a cat in Russia, but no clinical data are available (https://www.oie.int/en/scientific-expertise/specific-information-and-recommendations/questions-and-answers-on-2019novel-coronavirus/events-in-animals/). Probably, all these cases are also the result of contagions from their owners. Based on these results, it is possible that the number of affected cats living with COVID-19 owners is greater than that published to date, since these animals may be asymptomatic and not detected.

We consider that the limited number of animals included in our study can be a bias for the results. Nevertheless, the exceptional circumstances lived in Spain and in our region (La Rioja) during the sampling period (lockdown, confinement, quarantine, healthcare staff's work overload, high mortality rates and health system on the brink of collapse) make these data relevant. Our finding indicates that cats can contract the virus from infected humans, although the transmission of SARS-CoV-2 from animals to humans seems unlikely (https://www.who.int/es/emergencies/diseases/novel-coronavirus-2019/advice-for-public/q-a-coronaviruses). To date, all cases seem to be isolated ones related with human transmission through COVID-19-infected people taking care of the animals, without any epidemiological significance and considering cats as dead-end hosts (https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/COV-19/Bruschke_CVOs_Mink_SARS_CoV2_15May2020.pdf).

As a universal standard, hygiene measures should be exercised when living with a pet, especially if an infection is suffered. Currently, CDCs recommend restricting contact of people infected with COVID-19 with their companion animals (https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/COV-19/Bruschke_CVOs_Mink_SARS_CoV2_15May2020.pdf). At the same time, the OIE (https://www.oie.int/en/scientific-expertise/specific-information-and-recommendations/questions-and-answers-on-2019novel-coronavirus/) highly recommends to keep positive animals isolated from other unexposed ones.

4 | ADDENDUM

At the time we were reviewing this version of our manuscript, SARS-CoV-2 outbreaks on mink farms have been communicated in Spain and in other European countries. In the Netherlands, cats living in SARS-CoV-2-infected mink farms have showed antibodies against the virus (https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/COV-19/Bruschke_CVOs_Mink_SARS_CoV2_15May2020.pdf). The role of cats in these outbreaks related to minks is unknown. Further research from a one health perspective is needed to clarify these aspects.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

IRA: Ignacio Ruiz Arrondo; AP: Aránzazu Portillo; AMP: Ana María Palomar; SS: Sonia Santibáñez; PS: Paula Santibáñez; CC: Cristina Cervera; JAO: José A. Oteo.

IRA and JAO conceived and coordinated the study. IRA, AMP, SS, PS, CC and AP carried out the methodology. AP, IRA and AMP wrote the original draft. All authors reviewed, modified and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The manuscript has been sent to the pre-print server medRxiv (https://doi.org/10.1101/2020.05.14.20101444).

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