Received: 22 January 2021 | Revised: 3 April 2021 | Accepted: 10 May 2021

DOI: 10.1111/tbed.14153

SHORT COMMUNICATION

Transboundary and Emerging Diseases WILEY

No molecular evidence of SARS-CoV-2 infection in companion animals from Veracruz, Mexico

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Funding information
Consejo Nacional de Ciencia y Tecnología, Grant/Award Number: 312224

Abstract

Active epidemiological surveillance of infectious agents represents a fundamental tool for understanding the transmission dynamics of pathogens and establishing public policies that can reduce or limit their expansion. Epidemiological surveillance of emerging agents, such as the recently recognized severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of COVID-19, is essential to establish the risk of transmission between species. Recent studies reveal that companion animals are organisms susceptible to being infected by this pathogen due to the close contact they have with their owners. For this reason, the aim of the present work was to detect the presence of SARS-CoV-2 in dogs and cats in the state of Veracruz, Mexico, where there is active transmission of this microorganism in human populations. Oral and nasopharyngeal swab samples were collected from dogs and cats with a history of exposure to patients with COVID-19. Total RNA was extracted and detection of viral genes N1 and N2 was performed by reverse transcription polymerase chain reaction (RT-qPCR). All 130 samples of companion animals tested by RT-qPCR for SARS-CoV-2 were negative at the time they were collected. This study represents the second active surveillance of SARS-CoV-2 in populations of domestic dogs and cats in Latin America and the first approach in Mexico. Given that coronaviruses have shown a high capacity to be transmitted between species, it is imperative to establish measures to prevent this agent from entering and establishing in populations of companion animals.

KEYWORDS

cats, dogs, epidemiological surveillance, SARS-CoV-2, viral infection

1 | INTRODUCTION

COVID-19 is an emerging infectious disease caused by a recently identified zoonotic viral agent, called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The SARS-CoV-2, originally detected in Wuhan, China, has spread globally and was declared a pandemic agent by the World Health Organization on 11 March 2020 (Contini et al., 2020). On 29 February 2020, the first imported

case of COVID-19 was recorded in Mexico City; however, community transmission was not recognized in the country until 24 March 2020 (DGE, 2020). Since then, 1,313,675 confirmed cases have been registered, with a cumulative incidence rate of 1,037.84 cases per 100,000 inhabitants until week 51 of the current year (DGE, 2020).

In recent months, a potential process of reverse transmission of SARS-CoV-2 from humans to other species has been raised globally, for which the need to implement active epidemiological surveillance in other species, particularly those that have a close contact with humans, such as cats and dogs, has become imperative (Newman et al., 2020). Experimental studies have revealed that SARS-CoV-2 has a low replicative potential in dogs; however, cats are permissive to be infected through airway transmission (Shi et al., 2020). Additionally, in silico studies support the susceptibility of felines to this infectious agent (Martínez-Hernández et al., 2020). Reports of naturally infected companion animals have emerged from six countries in Europe, two from America and one from Asia (Table 1). In these countries, a prevalence that fluctuates between 0% and 17.65% for cats and 0% and 13.33% for dogs, has been recorded, depending on the detection method implemented (serology and/or molecular methods) (Table 1).

Most of the reports originate from animals without clinical signs; however, in four case studies carried out in cats, the presence of respiratory symptoms of varying severity, accompanied by lethargy and/or pyrexia, has been reported (Barrs et al., 2020; Garigliany et al., 2020). In the case of dogs, there is a worldwide debate regarding their susceptibility because there are no clear clinical signs suspicious for the condition; thus, the possible impact that this viral agent may have on dog populations remains unknown (Segalés et al., 2020; Sit et al., 2020). These data highlight the necessity to improve an active surveillance study of the circulation of SARS-CoV-2 in companion animals in Mexico.

In the state of Veracruz, there have been 41,816 cases of COVID-19 with an accumulative incidence rate of 492.62 cases per 100,000 inhabitants until week 51 of 2020 (DGE, 2020). The aim of this work was to monitor SARS-CoV-2 in companion animals in close contact with human patients with SARS-CoV-2 in Veracruz, one of the states with active transmission of COVID-19 in Mexico.

2 | MATERIAL AND METHODS

This study was approved by the Ethics and Research Committee of the Medical Faculty of the Universidad Nacional Autónoma de México (UNAM) (FM/DI/026/2020) and by the animal care and use committee of the School of Veterinary Medicine, Universidad Veracruzana, in Veracruz, Mexico.

A cross-sectional, observational epidemiological study was carried out in five municipalities of the state of Veracruz with active transmission of SARS-CoV-2 in human populations. To improve that, an intentional search was conducted for cats and dogs of owners having a confirmed diagnosis of COVID-19 (breathing and/or digestive symptoms, positive rapid test (exclusively viral antigen detection) and/or positive polymerase chain reaction test) 2 weeks prior to sampling. In addition, demographic data of companion animals (sex and age) were collected.

Dogs were physically restrained and cats were sedated with an intramuscular injection of ketamine and xylazine (Wildlife Pharmaceutics Mexico, Mexico City, Mexico). Nasal and/or oral swab samples were collected and fixed in 1.5 ml of Trizol Reagent (Invitrogen, California, USA) for inactivation of the virus and preservation of the viral RNA (Fernández-Figueroa et al., 2016). Afterwards, the samples were preserved in a cold chain until their extraction in the laboratory. 1 ml of the sample was transferred to a conical 1.5-ml plastic tube, after which 200 µl of cold chloroform was added (Sigma). This solution was mixed and centrifuged at 19,357× *g* for 10 min at 4°C. The aqueous phase was recovered and 500 µl of cold isopropanol was added (Sigma). The resulting solution was mixed for 15 s and incubated overnight at −20°C. Thereafter, the solution was centrifuged at 19,357× *g* for 10 min at 4°C. The supernatant was discarded and 1 ml ethanol 80% (Sigma) was added, the solution was mixed for 10 s and centrifuged at 19,357× *g* for 10 min at 4°C. The ethanol was discarded, the excess was air-dried, and the pellet was suspended in 40 µl RNase free water. The total RNA was quantified using a NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) and subsequently adjusted to an average concentration of 5 ng. The extracted RNA samples were subjected to cDNA synthesis using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, California, USA). To confirm the presence and integrity of the extracted RNA, dog-specific cytochrome oxidase subunit 1 (*COX-1*) amplification was performed (Sales et al., 2020). In the case of cats, after cDNA synthesis, to check the RNA quality, PCR was performed for the endogenous control, *COX-1*, by conventional PCR (cPCR) using the primers and conditions of Hafner et al. (1994).

For SARS-CoV2 detection, we amplified de nucleocapsid genes N1 and N2 (Cat. 10006770. 2019-nCov CDC EUA Kit, IDT), using GoTaq Probe 1-Step RT-qPCR System (Cat. A6121, Promega) in a reaction volume of 20 µl containing: 10 µl GoTaq, 1.5 µl probe (N1, N2), 4 µl GoScript, 3.1 µl Nuclease-Free water and 5 µl RNA. As positive control, Synthetic DNA Gen N of SARS-CoV2 (Cat. 10006625. 2019-nCoV_N_ Positive Control, IDT) was used. The thermal profile was as follows: 45°C during 15 min, 95°C during 2 min and 40 cycles at 95°C for 15 s and 60°C for 1 min. The 7500 FAST Real-Time PCR System was used. Data were analysed according to CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.

3 | RESULTS

During the period from 1 October to 18 December of 2020, a total of 130 samples of companion animals (100 canines and 30 felines) were collected from 15 municipalities in three regions of the state of Veracruz. The localities with the largest number of collected samples were Veracruz (33) followed by Tuxpan (30). The municipalities with the least number of samples were Xalapa (3) and Poza Rica (2), while

TABLE 1 SARS-COV-2 detection studies in cats and dogs worldwide

Species	Detection method	Analysed animals	Positive animals	Prevalence	Country	Sample period	Reference
Cat	RT-qPCR (Neutralization test)	1	1	-	Belgium	March, 2020	Garigliany et al., 2020
	RT-qPCR (Viral sequencing)	1	1	-	Brazil	Not reported	Carlos et al., 2021
	RT-qPCR (ELISA test, Viral sequencing and Viral isolation)	17	3	17.65	Chile	May and September, 2020	Neira et al., 2020
	RT-qPCR (Viral sequencing and Neutralization test)	50	6	12.00	China	February–August, 2020	Barrs et al., 2020
	ELISA	102	15	14.71	China	January–March, 2020	Zhang et al., 2020
	Neutralization test	102	11	10.78	China	January–March, 2020	Zhang et al., 2020
	Neutralization test	131	1	0.76	Croatia	February–June, 2020	Stevanovic et al., 2020
	Luciferase Immunoprecipitation Systems (LIPS)	9	0	0.00	France	March, 2020	Temmam et al., 2020
	RT-qPCR (Viral sequencing)	22	1	4.55	France	April, 2020	Sailleau et al., 2020
	ELISA (Indirect immunofluorescence test (iIFT) and Neutralization test)	920	6	0.65	Germany	April–September, 2020	Michelitsch et al., 2020
	RT-qPCR	314	0	0.00	Italy	March and May, 2020	Patterson et al., 2020
	Neutralization test	191	11	5.76	Italy	March and May, 2020	Patterson et al., 2020
	RT-qPCR (Sequencing)	1	1	-	Italy	None specified	Musso et al., 2020
	RT-qPCR (ELISA, Neutralization test and Viral sequencing)	1	1	-	Spain	March–April, 2020	Segalés et al., 2020
	RT-qPCR	8	1	12.50	Spain	April–May, 2020	Ruiz-Arrondo et el. 2020
	RT-qPCR	2	2	-	US	March–April, 2020	Newman et al., 2020
	RT-qPCR (Viral isolation, Viral sequencing, and Neutralization test)	17	3	17.65	US	June–July, 2020	Hamer et al., 2020
	ELISA/Neutralization test	10		0.00	US	None specified	Kim et al., 2020
Species	Detection method	Analysed animals	Positive animals	Prevalence	Country	Sample period	Reference
Dog	RT-qPCR (ELISA test)	10	0	0.00	Chile	May and September, 2020	Neira et al., 2020
	RT-qPCR (ELISA, Neutralization test, Viral sequencing, Viral isolation)	15	2	13.33	China	February–March, 2020	Sit et al., 2020
	Neutralization test	654	2	0.31	Croatia	February–June, 2020	Stevanovic et al., 2020
	ELISA	172	13	7.56	Croatia	February–June, 2020	Stevanovic et al., 2020
	Luciferase Immunoprecipitation Systems (LIPS)	12	0	0.00	France	March, 2020	Temmam et al., 2020
	RT-qPCR	11	0	0.00	France	April, 2020	Sailleau et al., 2020
	RT-qPCR	180	0	0.00	Italy	March and May, 2020	Patterson et al., 2020
	Neutralization test	451	15	3.33	Italy	March and May, 2020	Patterson et al., 2020
	RT-qPCR	12	0	0.00	Spain	April–May, 2020	Ruiz-Arrondo et al., 2020
	RT-qPCR (Viral isolation and Neutralization test)	59	1	1.69	US	June–July, 2020	Hamer et al., 2020
	ELISA (Neutralization test)	96		0.00	US	None specified	Kim et al., 2020

Otatitlan, Papantla and Texistepec only registered a single sample each (Table 2).

Regarding sex, in the case of canines, 63 samples were collected from males and 37 from females, while in the case of felines, samples were taken from 13 males and 17 females. The ages of the sampled animals ranged from one month to 14 years, with an average of 5 years (Table 2).

RNA quality was evaluated by the amplification of the endogenous *COX*-1 gene from all tested samples. All 139 samples of companion animals tested by RT-qPCR for SARS-CoV-2 were negative at the time they were collected.

4 | DISCUSSION

This study represents the second active surveillance of SARS-CoV-2 in populations of domestic dogs and cats in Latin America and the first approach in Mexico. In the present study, the presence of SARS-CoV-2 was not detected in companion animals in the state of Veracruz, Mexico, which is consistent with results of other studies that have implemented molecular methods (e.g. RT-qPCR) for the detection of this agent in Chile, Italy and Spain (Neira et al., 2020; Patterson et al., 2020; Ruiz-Arrondo et al., 2020).

Previous studies in cats from Belgium, Brazil, China and the USA have demonstrated the presence of SARS-COV-2 RNA in nasopharyngeal and rectal samples from animals whose owners had been diagnosed with COVID-19 between four and 15 days prior to collection of the sample or the manifestation of clinical signs (Barrs et al., 2020; Garigliany et al., 2020; Hamer et al. 2020). In the case of dogs, the only record from the USA that reports exposure time from the owner's confirmation positive for COVID-19 and the positive test of the animal was seven days (Hamer et al., 2020). A study in Italy where samples were collected from dogs and cats with owners confirmed with COVID-19, 15 days before taking the sample, presented similar results to those of our study, where all the samples were negative (Patterson et al., 2020). It is important to mention that the two-week collection period can be an important factor in the negative samples obtained, as the window period in which RT-PCR is effective in detecting SARS-COV-2 infection in companion dogs and cats is not clear. For this reason, negative results should be analysed with caution.

In the case of the duration of the positive samples by RT-PCR, it has been shown in a single study in Chile that the positivity is variable as in the case of a female cat that remained with a positive RT-PCR test in faeces for 17 days and other two animals from the same study that presented positive tests only on days five and eight (Neira et al., 2020).

Most of the studies in which positive animals have been reported were done by serological methods, such as microneutralization and/or ELISA (Michelitsch et al., 2020; Patterson et al., 2020; Segalés et al., 2020; Stevanovic et al., 2020; Zhang et al., 2020), where positive sera were shown to present cross-reactivity with other viral agents of community transmission, such as feline coronavirus (FCoV) (Kim et al., 2020). These results have important implications in the monitoring of SARS-CoV-2 exposure in companion animals, as cross-reactivity can generate a confounding effect in animal populations with high circulation of other coronaviruses.

TABLE 2 Characteristics of pets screened for SARS-CoV-2 during October–December 2020), in Veracruz, Mexico

Municipality	Collection period	Collected animals	Species Cats	Species Dogs	Ages Cats	Ages Dogs
Acayucan	November–December, 2020	23	1 (1F)	22 (17F, 5 M)	1 year	5 months–16 years
Álamo Temapache	October–November, 2020	7	2 (2F)	5 (4F, 1 M)	5 months–5 years	1 month–5 years
Cerro Azul	October–November, 2020	7	-	7 (7F)	-	11 months–5 years
Coatepec	November, 2020	5	-	5 (3F, 2 M)	-	4 months–11 years
Jesús Carranza	October, 2020	2	-	2 (1F, 1 M)	-	2–9 years
Oluta	November, 2020	3	-	3 (2F, 1 M)	-	2–5 years
Orizaba	October, 2020	4	-	4 (1F, 3 M)		6–8 years
Otatitlan	November, 2020	1	1 (1 M)	-	1 year	-
Papantla	December, 2020	1	-	1 (1 M)	-	1 year
Poza Rica	November–December, 2020	3	2 (2 M)	1 (1 M)	2 years	6 years
Soconusco	December, 2020	7	-	7 (4F, 3 M)	-	2–9 years
Texistepec	November, 2020	1	-	1 (1 M)	-	6 years
Tuxpan	October–December, 2020	30	20 (9F, 11 M)	10 (2F, 8 M)	5 months–5 years	7 months–8 years
Xalapa	November, 2020	3	2 (1F, 1 M)	1 (1F)	6–7 years	14 years
Veracruz	October–December, 2020	33	2 (2 M)	31 (21F, 10 M)	1 year	3 months–7 years
Total	October–December, 2020	130	30 (13F, 17 M)	100 (63F, 37 M)	5 months–7 years	1 month–16 years

F: Female; **M**: Male.

The reports in which the presence of SARS-CoV-2 has been detected in cats and dogs show that close contact between infected humans poses a risk to companion animals (Barrs et al., 2020; Garigliany et al., 2020; Segalés et al., 2020). Particularly, Brazil was the first country in Latin America to report the sequencing of the complete SARS-COV-2 genome from a cat infected by its owner (Carlos et al., 2021).

For this reason, the need to limit contact between human patients, with a presumptive or confirmatory diagnosis of COVID-19, and their companion animals is reiterated, in an effort to avoid infecting these companion species through airborne transmissions. Historically, it has been documented that humans can infect companion animals with other infectious agents, such as tuberculosis in companion dogs (Erwin et al., 2004; Hackendahl et al., 2004).

Given that coronaviruses have shown a high capacity to be transmitted between species, it is imperative to establish measures to prevent this agent from entering and establishing in populations of companion animals (Contini et al., 2020; Martínez-Hernández et al., 2020). Additionally, it is essential for owners to remember that pets are not a source of SARS-CoV-2 infection for humans, so their safety and integrity must be preserved, and owners should avoid abandoning them. The participation of veterinarians in the surveillance of this emerging agent is essential, which will guide health policies to safeguard the health and well-being of companion animals during this global emergency.

ACKNOWLEDGEMENTS

We thank the dog and cat owners who decided to participate in this project. We also thank David Reyes-Córdova for field assistance. The project was financed by CONACYT 312224 'COVID-19 en México: epidemiología de la enfermedad en población mexicana con énfasis en pacientes oncológicos e identificación de animales de compañía como unidades centinela para detectar SARS-CoV-2'.

CONFLICT OF INTEREST

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, non-financial interest in the subject matter or materials discussed in this manuscript.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Animals were handled according to National Legislation and Ethics and with approval of Research Committee of the Medical Faculty of the Universidad Nacional Autónoma de México (UNAM) (FM/DI/026/2020).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Sánchez-Montes S, Ballados-González GG, Gamboa-Prieto J, et al. No molecular evidence of SARS-CoV-2 infection in companion animals from Veracruz, Mexico. *Transbound Emerg Dis*. 2022;69:2398–2403. https://doi.org/10.1111/tbed.14153