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 SARS-CoV2 from humans to 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). In silico studies support the susceptibility of felines to SARS-CoV-2 from humans to 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).

Recently, several reports of naturally infected companion animals have been published in Europe, Latin America, and Asia (Table 1). These data highlight the necessity to improve active surveillance studies 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 Pharmaceuticals 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 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...
| 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       | 17               | 3                | 17.65      | Chile            | May and September, 2020| Neira et al., 2020         |
|         | isolation)                                            |                  |                  |            |                  |                        |                            |
|         | RT-qPCR (Viral sequencing and Neutralization test)    | 50               | 6                | 12.00      | China            | February–August, 2020  | Bars 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            | Saleau et al., 2020       |
|         | ELISA (Indirect immunofluorescence test (IFT) and     | 920              | 6                | 0.65       | Germany          | April–September, 2020  | Michelitsch et al., 2020  |
|         | Neutralization test                                   |                  |                  |            |                  |                        |                            |
|         | 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         | 1                | 1                | -          | Spain            | March–April, 2020      | Segalés et al., 2020      |
|         | sequencing)                                           |                  |                  |            |                  |                        |                            |
|         | RT-qPCR                                               | 8                | 1                | 12.50      | Spain            | April–May, 2020        | Ruiz-Arrondo et al., 2020 |
|         | RT-qPCR                                               | 2                | 2                | -          | US               | March–April, 2020      | Newman et al., 2020       |
|         | RT-qPCR (Viral isolation, Viral sequencing, and       | 17               | 3                | 17.65      | US               | June–July, 2020        | Hamer et al., 2020        |
|         | Neutralization test                                   |                  |                  |            |                  |                        |                            |
| Dog     | ELISA/Neutralization test                             | 10               | 0                | 0.00       | US               | None specified         | Kim 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            | Saleau 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                | 0.00       | US               | None specified         | Kim et al., 2020          |
Otalitlan, 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.

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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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