UNIVERSIDAD DE CÓRDOBA

FACULTAD DE MEDICINA VETERINARIA Y ZOOTECNIA

CARACTERIZACIÓN DE LA INFECCIÓN POR SARS-CoV-2 EN PERROS Y GATOS EN 8 MUNICIPIOS DEL DEPARTAMENTO DE CÓRDOBA, COLOMBIA.

Yesica Botero Serna

TESIS DE MAESTRÍA EN CIENCIAS VETERINARIAS DEL TRÓPICO

DIRECTOR

CATY MILENA MARTÍNEZ BRAVO

Biólogo M.Sc.

CO-DIRECTOR

ALFONSO CALDERON RANGEL

MVZ, D.Sc.

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Nota de aceptación

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RESUMEN

Antecedentes. La COVID-19 es una enfermedad emergente de posible origen zoonótico causada por el virus SARS-CoV-2. Se presume que ésta tuvo su origen en un animal y de este pasó a los seres humanos, en los cuales se ha propagado con mucho éxito desde que fue descubierto por primera vez a finales de 2019. Sin embargo, se ha demostrado su retorno de humanos a otras especies animales y nuevamente de regreso a las personas, lo que ha implicado diversas mutaciones y variantes que han afectado la dinámica de la infección por SARS-CoV-2. Las posibles vías de transmisión y su potencial zoonótico son actual objeto de investigación a nivel mundial, no obstante, en Colombia la situación epidemiológica de esta patología no puede ser estimada con precisión debido a la baja notificación de casos humanos y animales, y a los sistemas de vigilancia epidemiológica fragmentados. Por lo tanto, llevar a cabo estrategias de lucha contra las zoonosis, como la vigilancia de enfermedades zoonóticas emergentes, beneficiaría a la región y al país con una toma de conciencia y comprensión de las sinergias implicadas bajo el enfoque “Una Salud”. **Objetivo.** Caracterizar la infección por SARS-CoV-2 en perros y gatos en 8 municipios del departamento de Córdoba, Colombia. **Metodología.** Con la aprobación del comité de ética del Instituto de Investigaciones Biológicas del Trópico con No. 0410-2020 y del comité de ética de la facultad de Medicina Veterinaria y Zootecnia de la Universidad de Córdoba Acta No. 005 26 (May 2021). Se realizó una vigilancia molecular y serológica descriptiva de corte transversal, donde se tomaron muestras de hisopados orales y rectales, y sangre de forma proporcional a la población estimada en perros y gatos de los 8 municipios del departamento utilizando estrategias de muestreo casa a casa por búsqueda, campañas de desparasitación y vacunación con aliados departamentales y centros veterinarios y petición directa de tutores responsables entre septiembre de 2020 a septiembre de 2021. **Resultados.** El presente trabajo bajo el enfoque de una salud aportó el primer reporte genómico de infección por linaje B.1.111 de SARS-CoV-2 en un gato en Colombia y el primer evento de transmisión humano-perro de la variante IOTA en Latinoamérica. Se identificaron 4 casos activos y una tasa de infección del 11,5%. Se generaron 3 artículos científicos, 2 presentados en una revista Q2 y una Q1 en inglés, y uno que será sometido en una revista Q2 en español donde se plasmaron e hicieron públicos los datos obtenidos a partir de la presente investigación. **Conclusión.** La caracterización de la infección por SARS-CoV-2 en animales domésticos viviendo en el departamento de Córdoba aportó información relevante para motivar nuevas investigaciones implementando la interdisciplinariedad y diferentes métodos serológicos, moleculares y genómicos de vigilancia epidemiológica.

Palabras clave. Vigilancia epidemiológica, salud pública, Una Salud, zoonosis virales, COVID-19

ABSTRACT

Background. COVID-19 is an emerging disease of possible zoonotic origin caused by the SARS-CoV-2 virus. It is presumed to have originated in an animal and passed from that animal to humans, in which it has spread very successfully since it was first discovered in late 2019. However, its return from humans to other animal species and again back to humans has been demonstrated, which has involved several mutations and variants that have affected the dynamics of SARS-CoV-2 infection. The possible routes of transmission and its zoonotic potential are currently under investigation worldwide; however, in Colombia, the epidemiological situation of this pathology cannot be accurately estimated due to the low notification of human and animal cases and fragmented epidemiological surveillance systems. Therefore, carrying out strategies to control zoonoses, such as emerging zoonotic disease surveillance, would benefit the region and the country with an awareness and understanding of the synergies involved under the "One Health" approach. **Objective.** To characterize SARS-CoV-2 infection in dogs and cats in 8 municipalities of the department of Córdoba, Colombia. **Methodology.** With the approval of the ethics committee of the Instituto de Investigaciones Biológicas del Trópico (IIBT) No. 0410-2020 and the ethics committee of the Faculty of Medicina Veterinaria y Zootecnia of the Universidad de Córdoba Acta No. 005 26 (May 2021). A cross-sectional descriptive molecular and serological surveillance was carried out, where oral and rectal swabs and blood samples were taken proportionally to the estimated population of dogs and cats in the 8 municipalities of the department using the following strategies: house-to-house sampling, deworming, and vaccination campaigns with departmental partners and veterinary centers, and direct request between September 2020 and September 2021. **Results.** Under the one health approach, this study provided the first genomic report of SARS-CoV-2 B.1.111 lineage infection in a cat in Colombia and the first human-dog transmission event of the IOTA variant in Latin America. Four active cases and an infection rate of 11.5% were identified. Three scientific articles were generated, two presented in a Q2 and a Q1 journal in English, and one to be submitted to a Q2 journal in Spanish where the data obtained from the present investigation were presented and made public. **Conclusion.** The characterization of SARS-CoV-2 infection in domestic animals living in the department of Córdoba provided relevant information to motivate further research by implementing interdisciplinary and different serological, molecular, and genomic methods of epidemiological surveillance.

Keywords. Surveillance, Public Health, One Health, viral zoonoses, COVID-19

INTRODUCCIÓN

La situación epidemiológica de las zoonosis en Colombia no puede ser estimada con precisión porque el panorama de las zoonosis está caracterizado por: baja notificación de casos humanos de zoonosis (1), falta de una política de salud pública veterinaria, falencias en la legislación en cuanto a la actualización del panorama epidemiológico de las zoonosis, los sistemas de vigilancia epidemiológica fragmentados y la falta de una red de laboratorios de diagnóstico (1–4).

Las enfermedades zoonóticas plantean una importante amenaza para la salud pública humana y por lo tanto deben ser tenidas en cuenta por ser causantes de enfermedades graves, como la actual pandemia por la enfermedad COVID-19 causada por el coronavirus SARS-CoV-2 (4,5). Cuyo descubrimiento se dio como resultado de la vigilancia epidemiológica de enfermedades emergentes a nivel mundial. Es así como, en diciembre de 2019 se comenzaron a evidenciar casos en humanos de neumonía aguda con etiología desconocida en Wuhan, China (6). Para enero del siguiente año se logró aislar el agente etiológico de esta enfermedad emergente, siendo este betacoronavirus con posible origen zoonótico, denominado SARS-CoV-2 debido al síndrome respiratorio agudo que caracteriza a la enfermedad que causa en humanos o en sus siglas en inglés Severe Acute Respiratory Syndrome (6–8).

Debido a la fácil y rápida diseminación del virus a otros países del mundo, en marzo de 2020 se declaró un estado de pandemia y para su contención cada país tuvo que establecer distintas medidas de bioseguridad, incluido un periodo de cuarentena masiva y periodos de cuarentena intermitentes (9). En consecuencia, el comportamiento socioeconómico se vio afectado de forma negativa, forzando a muchas personas a una convivencia estrecha con su núcleo familiar. Asimismo, el contacto con las mascotas aumentó y esto afectó de forma positiva el incremento de casos en animales domésticos, siendo los gatos la segunda especie que más casos ha reportado a nivel global (10).

Al mes de junio de 2022, se han reportado más de 300 casos de mamíferos no humanos infectados de forma natural con SARS-CoV-2 a nivel mundial (10), no obstante, los reportes de infección en animales sólo serían la punta del iceberg de los posibles casos de SARS-CoV-2 en especies animales diferentes al hombre. Menos de 180 secuencias genómicas de SARS-CoV-2 en perros y gatos han sido reportados en el Global Initiative on Sharing All Influenza Data (GISAID) de los cuales sólo unos pocos reportes publicados han incluido un análisis a profundidad de las secuencias genómicas virales que relacionan a los gatos infectados con su tutor responsable (11–13) y ninguno de estos había sido reportado en Colombia, un país ubicado en el trópico que ha sido fuertemente golpeado por esta pandemia reportando al mes de junio de 2022 más de 6 millones de casos humanos y más de 139 mil fallecidos por esta enfermedad (14).

Hasta el momento solo los felinos, hámsteres, venados de cola blanca y mustélidos han mostrado contagios entre individuos de su misma especie, sin embargo, sólo

se ha demostrado el retorno del virus hacia los humanos en poblaciones de visones de granja y hámsteres (7,10,15). Donde, se demostró la existencia de mutaciones de interés involucradas en estos eventos de salto humano-animal-humano del virus (15–19). Un estudio reciente sugiere un evento de transmisión humano-gato humano (20), sin embargo, aún se desconoce el papel que cumplen los animales en la dinámica de la infección por SARS-CoV-2 (7,21). Por lo tanto, se deben intensificar los esfuerzos en la búsqueda de casos en animales, dado que, esto nos permitiría mejorar la vigilancia genómica de los saltos o mutaciones que puedan promover una mayor patogenicidad de este coronavirus. Lo que implica procesos de planificación, planes estratégicos, aunado a marcos de seguimiento y evaluación relacionados con la vigilancia de esta enfermedad bajo el enfoque One Health (22,23).

En Colombia Los Objetivos de Desarrollo Sostenible (ODS) adoptan un enfoque integrado, haciendo hincapié en la equidad, la sostenibilidad y la adopción de un enfoque multisectorial “Una Salud” para las enfermedades zoonóticas que aborde la interconexión de la salud y sus factores determinantes de carácter social y económico (24,25). Por lo tanto, llevar a cabo estrategias de lucha contra las enfermedades zoonóticas, como la vigilancia de enfermedades zoonóticas emergentes, beneficiaría a la región y al país con una toma de conciencia y comprensión mayores de las sinergias entre las enfermedades zoonóticas, el enfoque “Una Salud” y los ODS (23).

Esto se ve reflejado en el Plan de Desarrollo Departamental 2020 – 2023 del departamento de Córdoba donde se resalta el papel que juega el diagnóstico de enfermedades dentro del componente “Salud para el bienestar” (26). Por ende, el presente trabajo de grado realizó una caracterización de la infección por SARS-CoV-2 en perros y gatos que tuvieron o no contacto establecido con personas positivas a COVID-19 del departamento de Córdoba.

OBJETIVOS

Objetivo general

Caracterizar la infección por SARS-CoV-2 en perros y gatos en ocho municipios del departamento de Córdoba.

Objetivos específicos

- Diagnosticar casos activos de SARS-CoV-2 en perros y gatos en el departamento de Córdoba.
- Detectar las variantes presentes en el genoma del virus SARS-CoV-2 circulante en perros y gatos en el departamento de Córdoba.
- Determinar la tasa de infección de SARS-CoV-2 en perros y gatos en el departamento de Córdoba.
- Aumentar la transferencia de conocimiento interinstitucional y comunitario encaminado a la implementación de acciones para la prevención y control de agentes biológicos de alto riesgo para la salud humana.

ARTÍCULOS GENERADOS

CAPÍTULO 1

En cumplimiento al primer y segundo objetivo del presente trabajo se publicó el artículo titulado "**First report and genome sequencing of SARS-CoV-2 in a cat (*Felis catus*) in Colombia"** en la revista Q2 MEM INST OSWALDO CRUZ, RIO DE JANEIRO, VOLUME 117 | 2022 con DOI: 10.1590/0074-02760210375.

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First report and genome sequencing of SARS-CoV-2 in a cat (*Felis catus*) in Colombia

Yesica Botero[1], Juan David Ramírez[2], Héctor Serrano-Coll[3], Ader Aleman[1], Nathalia Ballesteros[2], Caty Martinez[1], Marina Muñoz[2], Alfonso Calderon[1], Luz H Patiño[2], Camilo Guzman[1], Sergio Castañeda[2], Yonairo Hererra[1], Salim Mattar[1]/+

[1]Universidad de Córdoba, Instituto de Investigaciones Biológicas del Trópico, Montería, Colombia
[2]Universidad del Rosario, Facultad de Ciencias Naturales, Centro de Investigaciones en Microbiología y Biotecnología-UR, Bogotá, Colombia
[3]Universidad CES, Instituto Colombiano de Medicina Tropical, Medellín, Colombia

BACKGROUND Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a virus of zoonotic origin that can bind to ACE2 receptors on the cells of many wild and domestic mammals. Studies have shown that the virus can circulate among animals mutate, lead to animal-to-human zoonotic jump, and further onward spread between humans. Infection in pets is unusual, and there are few human-to-pet transmission reports worldwide.

OBJECTIVE To describe the SARS-CoV-2 infection in a domestic animal in Córdoba, Colombian Caribbean region.

METHODS A cross-sectional molecular surveillance study was carried out, oral and rectal swabs were taken from cats and dogs living with people diagnosed with coronavirus disease 2019 (COVID-19).

RESULTS SARS-CoV-2 was found in a cat living with a person with COVID-19. Genome sequencing showed that the B.1.111 lineage caused the infection in the cat. The owner's sample could not be sequenced. The lineage is predominant in Colombia, and this variant is characterised by the presence of the D614D and Q57H mutation.

CONCLUSION The present work is the first report of an infected cat with SARS-CoV-2 with whole-genome sequencing in Colombia. It highlights the importance of detecting SARS-CoV-2 mutations that could promote the transmissibility of this new coronavirus. There is still a significant information gap on human-to-cat-to-human infection; we encourage self-isolation measures between COVID-19 patients and companion animals. The findings of this study give a preliminary view of the current panorama of SARS-CoV-2 infection in animals in Colombia.

Key word: population surveillance - public health - one health - COVID-19 - viral zoonoses

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) appears to be of zoonotic origin;[1] this new coronavirus can bind to ACE2 receptors in the cells of a wide variety of mammals.[1,2] The infection has been described in humans and animals such as cats, dogs, ferrets, hyenas, coatis, otters, big cats (lions, tigers, panthers, pumas, leopards), non-human primates, white-tailed deer, manatees, hippopotamuses, hamsters, and minks naturally infected.[3,4,5] To date, less than 400 cases describing animals infected with SARS-CoV-2 have been reported,[3] a few with genomic analyses,[3,6,7] and only one described in Colombia, a country located in the tropics that has been heavily hit by the pandemic.[8]

There are reports of humans that have transmitted the SARS-CoV-2 infection to animals. So far, only felines, rhesus macaque, mustelids, and Syrian hamsters have shown infection between individuals of the same species. The transmission of the virus from humans to big and small felines is evident, but the virus's return to humans has not yet been demonstrated.[6,7]

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+ Corresponding author: smattar@correo.unicordoba.edu.co
https://orcid.org/0000-0003-0526-4630
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The role that animals play in the dynamics of this infection is still unknown. However, some studies have shown that minks (*Neovison vison*) develop coronavirus disease 2019 (COVID-19), severe respiratory symptoms and death.[9] Researchers have described that the virus has managed to infect humans again, introducing mutations in its protein S such as Y453F, F486L, and N501T, which could be of interest since they are directly involved in the binding and internalisation of the virus in host cells.[9,10,11] Another study showed that the virus could circulate within hamsters and lead to human infections, as they described humans and hamsters infected with the Delta variant (AY.127).[4] It strongly suggests that some pets can potentially be a secondary reservoir of SARS-CoV-2.[4]

The "One Health" approach offers a better understanding of these human-animal-environment interactions and enables better planning and decision-making around the public health of this emerging disease. Therefore, this research aimed to describe the SARS-CoV-2 infection in a domestic animal in Córdoba, Colombian Caribbean. The present study contributes information and awareness of the current panorama of SARS-CoV-2 infection in cats in the country.

SUBJECTS AND METHODS

Ethics approval and consent to participate - The study follows the ethical standards of the Ministry of Health of Colombia Resolution No. 8430 of 1993. The

present study data corresponds to one patient coded under strict anonymity with an internal laboratory number. This study is the results of a research project that was approved by the Ethics Committee of the Institute of Biological Research of the Tropic of the University of Córdoba, with the number No. 0410-2020 and by the Ethics Committee of the Faculty of Veterinary Medicine Act No. 005 26 (May 2021). All people involved in the research approved the publication.

In a cross-sectional surveillance study, two hundred ninety samples were collected from domestic animals proportionally in eight municipalities of the Córdoba department located in the north-western Colombian Caribbean region. Informed consent was obtained from the pet's legal guardians for sample collection in this study. Oropharyngeal and rectal swabs were collected for felines and canines as a part of the genomic and epidemiologic surveillance of pets living with COVID-19 patients' households. Samples were conserved in a viral transport medium (VTM) for SARS-CoV-2 detection by reverse transcription real-time polymerase chain reaction (RT-qPCR).

A SARS-CoV-2 positive person who had signs and symptoms of COVID-19, like abdominal and chest pain, headache, and fever, alerted researchers from his cat, who died and was close to him. The cat died and showed respiratory manifestations 12 hours before. The animal was necropsied using the biosafety elements described for this procedure in animals suspected of SARS-CoV-2.[12,13,14] According to the Organización Mundial de Sanidad Animal (OIE) and the Centers for Disease Control and Prevention (CDC), oral and rectal swabs were taken from other animals living in the same household and near the deceased cat's home within a radius of approximately 500 metres.[13,14]

A surgical scalpel was used, a sample of different tissues (trachea, tonsil, and lungs) from the cat was macerated and homogenised within the viral transport medium. RNA extraction was performed in a BSL-2 laboratory with 200 µL of supernatant from each sample with the commercial GeneJET RNA Purification Kit™ (Thermo Scientific) based on lysis technology and silica-based membrane affinity columns following the manufacturer's instructions. RNA was used to detect SARS-CoV-2 by amplifying the envelope gene (E gene) using the Biorad CFX96™ RT-qPCR system and C1000 Touch™ thermocycler, following the protocol's primers and probes concentration recommendation published by Charité Hospital, Universitätsmedizin Berlin, Germany of Charité Berlin.[15] Using Primer E_Sarbeco_F (400 nm per reaction), E_Sarbeco_R2 (400 nm per reaction), Probe E_Sarbeco_P1 (200 nm per reaction), and 1-Step RT-qPCR Kit Plus ROX Vial (Thermo Scientific™) to prepare the master mix. For each reaction, 15 µL of the master mix and 5 µL of controls were added to this mixture. RT-qPCR cycling conditions were one cycle (50°C for 15 min), one cycle (95°C for 15 min) and then 45 cycles (95°C for 15 s), finally one cycle (60°C for 1 min). The sample was considered positive with a Cq ≤ 35. The gene library was prepared from the RNA extracted from the trachea, and the ARTIC network protocol (https://artic.network/ncov-2019) was used. Long-read Oxford Nanopore MinION sequencing was carried out using the MinKNOW application (v1.5.5). The raw Fast5 files were named base and demultiplexed using Guppy. Then, the reads were filtered, eliminating the possible chimeric reads. Finally, the genome assemblies were obtained following the MinION pipeline described in the ARTIC bioinformatics pipeline (https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html). All assemblies were made based on the PANGOLIN nomenclature lineage mapper.[16]

RESULTS

The cat owner was diagnosed by RT-qPCR (Cq 27.06) as COVID-19 with mild clinical manifestations. The cat was necropsied because of the diagnosis and samples were taken for RT-qPCR detection of SARS-CoV-2. Samples of the trachea (Cq 23.13), tonsil (Cq 32.59), and fecal swab (Cq 33.5) were positive to SARS-CoV-2 (Table I, Figure). The animal was neutered and vaccinated against rabies and lived with a 1-year-old dog and a 5-year-old cat. Both pets had outdoor access. The cat showed a history of scarring from fights with other domestic and feral animals. Samples were taken from the domestic animals living in the same household. Near the housing case, eleven cats and seven dogs' samples were also taken; all of them were negative to the RT-qPCR test for SARS-CoV-2 (Table II).

The tracheal purified RNA sample from the positive cat was sequenced, obtaining a genome sequence of the beta-coronavirus SARS-CoV-2 of the B.1.111 lineage (V. 2021-05-27), which presented mutations of interest previously described in Spike (D614G) and ORF3a (Q57H) and others that have not yet been described [Figure (B), Table III]. The owner's sample could not be sequenced.

Most of the 290 domestic animals evaluated in the present work were cats. RT-qPCR and successful genome sequencing detected SARS-CoV-2 in a cat (EPI_ISL2339859.2) and a dog (EPI_ISL8422346) in two different cities for a total active infection rate of 0.69% in the department of Córdoba, Colombia (Table IV).[8]

TABLE I

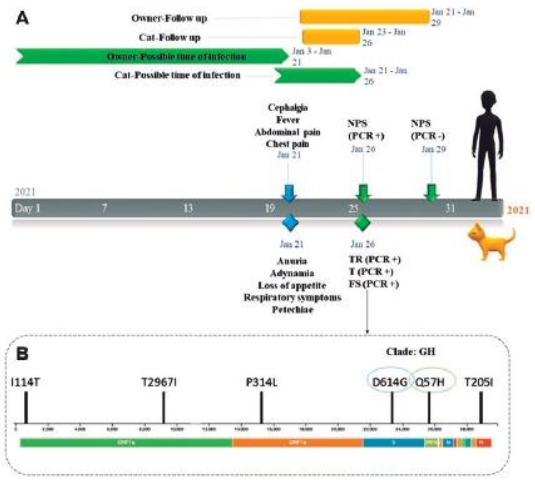
Reverse transcription real-time polymerase chain reaction (RT-qPCR) results of the samples from the cat and its owner

Sample owner (D/M/Y)	Result RT-qPCR (Cq)
NPS (26/01/21)	Positive (27,06)
NPS (29/01/21)	Negative (38,14)
Sample cat (D/M/A)	Result RT-qPCR (Cq)
Trachea (26/01/21)	Positive (23,13)
Tonsil (26/01/21)	Positive (32,59)
Fecal swab (26/01/21)	Positive (33,5)
Lung (26/01/21)	Negative (N/A)

NPS: nasopharyngeal swab.

DISCUSSION

A genome sequence of SARS-CoV-2 was obtained as reported by Ramírez et al.[16] from the trachea sample belonging to the B.1.111 lineage (V. 2021-05-27). Genome analysis showed previously described mutations of interest in Spike (D614G) and ORF3a (Q57H) (Figure). The sequence obtained was placed in the GISAID virtual platform (EPI_ISL_2339859.2) (Figure). The genomic analysis for SARS-CoV-2 showed that it belongs to the phylogenetic Nextstrain clade 20A, and its lineages are currently circulating in Colombia. The lineage in which this sequence was classified is the predominant in the department of Córdoba (Colombia) and was detected for the first time in March 2020 with the D614G substitution in protein Spike, which gives the virus greater transmissibility, infectivity and increases the viral load in the respiratory tract.[17] This mutation promotes an open conformation of the receptor binding domain (RBD), which facilitates the interaction of the SARS-CoV-2 Spike protein with ACE2 receptors.[16] Therefore, it could also facilitate better interaction and affinity of the SARS-CoV-2 RBD with the ACE2 receptors of domestic animals such as dogs or cats.



(A) Timeline of the diagnostic tests and onset of symptoms of the cat and its owner. The possible moment of contagion is speculative. NPS: nasopharyngeal swab; TR: trachea; T: tonsil; FS: fecal swab. (B) Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transcriptome.

TABLE II

Characteristics of domestic animals sampled that were in contact with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) positive cat

Variables	N = 18 (100%)
Species	
Feline	11 (61.1)
Canine	7 (38.9)
Positivity RT-qPCR	0

RT-qPCR: reverse transcription real-time polymerase chain reaction.

Another significant mutation is Q57H, which is a poorly studied mutation that could be related to the greater transmissibility of SARS-CoV-2. This mutation occurs on the ORF3a protein, a viporin capable of acting with an ion channel, thus promoting the release of viral genetic material in the host cell.[18] Therefore, Q57H would further stabilise the quaternary structure of this viporin, facilitating the improved release of viral particles by promoting pore formation in the host cell.[19] Therefore, this mutation should be studied in greater depth in the pathogenesis of SARS-CoV-2 in both humans and animals.

On the other hand, Renata et al.[6] implemented a genomic analysis to suggest that the transmission of SARS-CoV-2 from a person to their pet is likely. The feline infected with SARS-CoV-2 was found in Montería, Córdoba. Montería is the capital of the department where the study was carried out. According to the Instituto Nacional de Salud of Colombia,[20] it has a higher population density of pets and humans and a more significant number of reported COVID-19 in humans (51% cases and 47% deaths of the department).

Another study reported high seropositivity for COVID-19 in people from the department of Córdoba (40.8%).[21] Altogether a high density of pets and a high density of people with COVID-19 could increase the risk of transmission of SARS-CoV-2 to domestic animals.

Also, it should be noted that the population of domestic cats and dogs in the Colombian Caribbean region tends to street lifestyle, increasing the contact of pets with other animals and people, which increases the risk of transmission of this virus to other species. However, in this study, the animals in contact with the positive cat had negative results.

Finally, this is the first report of SARS-CoV-2 genome sequencing in a cat in Colombia. Relevant data that contributes to the understanding of the role played by domestic animals in the dynamics of infection was obtained. To date, some studies of natural and experimental infection in animals have shown that dogs, felines, primates, and farm animals such as minks have a greater susceptibility to this betacoronavirus.[22] However, birds such as chickens, ducks, geese, and quail, which have been associated with the transmission of multiple respiratory viruses, one of them the avian influenza virus A(H5N1), A(H7N9), do not appear to be susceptible and they act as intermediate hosts for SARS-CoV-2.[22,23] Still, the appearance of new mutations in the RBD segment of protein S could promote a greater susceptibility of this coronavirus to a more significant number of animals, promoting the appearance of new variants of SARS-CoV-2.

Therefore, it is still necessary to carry out other studies under the "One Health" approach, including detailed knowledge of the viral circulation in humans and animals[24] — using other methods such as serological tests to obtain more evidence about SARS-CoV-2 prevalence in animals.

TABLE III

Whole-genome sequencing results

Sequence	hCoV-19/cat/Colombia/COR-U04/2021|EPI_ISL_2339859.2
Clade	20A (GH)
Total mutations	13
Substitutions	C241T, C346T, T606C, C1060T, C3037T, C9165T, C10507T, T13180G, C14408T, C18877T, A23403G, G25563T, C28887T

TABLE IV

Sample size description and active infection rate	N = 290 (100%)
Species	
Cat	254 (87,6)
Dog	36 (12,4)
City	
Sahagún	29 (10)
Montelíbano	13 (4,5)
Lorica	25 (8,6)
Tierralta	23 (7,9)
Planeta Rica	39 (13,4)
Montería	97 (33,4)
San Antero	20 (6,9)
Cereté	44 (15,2)
Positive RT-qPCR/NGS	2 (0,69)

NGS: next-generation sequencing; RT-qPCR: reverse transcription real-time polymerase chain reaction.

Although there are few human-to-pet transmission reports worldwide, SARS-CoV-2 has a wide host range.[3,4,5] Therefore, we encourage self-isolation measures between COVID-19 patients and companion animals to prevent the appearance of zoonotic events that could lead to new mutations in the virus.

In conclusion, the findings of this study give a preliminary view of the current panorama of SARS-CoV-2 infection in animals in Colombia. The work contributes to a better understanding of the animal-human interface in the epidemiology of this disease.

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AUTHORS' CONTRIBUTION

All authors contributed equally to the manuscript's concept, design of the study, collection, analysis, and interpretation of the data. Besides, all authors did review the paper and approved it before submitting it. YB, AA, CM, YH, AC and CG collected epidemiological data; YB, JDR, SM, NB, MM, HS, LHP and SC analysed the data and edited the manuscript. All authors read and approved the final manuscript. The authors declare no competing interests.

REFERENCES

1. Hu B, Guo H, Zhou P, Shi Z-L. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol. 2021; 19(3): 141-54.
2. Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong J-C, Turner AJ, et al. Angiotensin-converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. Circ Res. 2020; 126(10): 1456-74.
3. OIE - Organización Mundial de Sanidad Animal. Available from: https://wahis.oie.int/#/home.
4. Yen H-L, Sit THC, Brackman CJ, Chuk SSY, Cheng SMS, Gu H, et al. Transmission of SARS-CoV-2 (variant Delta) from pet hamsters to humans and onward human propagation of the adapted strain: a case study. SSRN [Preprint]. 2022. Available from: http://dx.doi.org/10.2139/ssrn.4017393.
5. USDA - US Department of Agriculture. Confirmed cases of SARS-CoV-2 in animals in the United States. Animal and plant health inspection service. 2021. Available from: https://www.aphis.usda.gov/aphis/dashboards/tableau/sars-dashboard.
6. Renata CAS, Mariano APM, Maciel BM, Gadelha SR, de Melo Silva M, Belitardo EMMA, et al. First genome sequencing of SARS-CoV-2 recovered from an infected cat and its owner in Latin America. Transbound Emerg Dis. 2021; 68(6): 3070-4.
7. Sailleau C, Dumarest M, Vanhomwegen J, Delaplace M, Caro V, Kwasiborski A, et al. First detection and genome sequencing of SARS-CoV-2 in an infected cat in France. Transbound Emerg Dis. 2020; 67(6): 2324-8.
8. Rivero R, Garay E, Botero Y, Serrano-Coll H, Gastelbondo B, Muñoz M, et al. Human-to-dog transmission of SARS-CoV-2 Lota variant: should COVID-19 patients avoid close contact with their pets during illness? Research Square [Preprint]. 2022. Available from: https://doi.org/10.21203/rs.3.rs-821033/v1.
9. Zhou P, Shi Z-L. SARS-CoV-2 spillover events. Science. 2021; 371(6525): 120-22.
10. Welkers MRA, Han AX, Reusken CBEM, Eggink D. Possible host-adaptation of SARS-CoV-2 due to improved ACE2 receptor binding in mink. Virus Evol. 2021; 7(1): 2020-2.
11. Hayashi T, Yaegashi N, Konishi I. Effect of RBD mutation (Y453F) in spike glycoprotein of SARS-CoV-2 on neutralizing antibody affinity. bioRxiv [Preprint]. 2020. Available from: https://doi.org/10.1101/2020.11.27.401893.
12. CDC - Centers for Disease Control and Prevention. Infection control guidance. 2021. Available from: https://www.cdc.gov/coronavirus/2019-ncov/hcp/infection-control-recommendations.html#print.
13. OIE - Organización Mundial de Sanidad Animal. Consideraciones para el muestreo, las pruebas y la notificación de SARS-CoV-2 en animales. 2020. Available from: https://www.oie.int/fileadmin/Home/MM/E_Sampling_Testing_and_Reporting_of_SARS-CoV-2_in_animals_final_7May_2020.pdf.

14. CDC - Centers for Disease Control and Prevention. Biosafety for specimen handling. 2021. Available from: https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html.

15. Corman V, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020; 25(3): 1-8.

16. Ramírez JD, Muñoz M, Ballesteros N, Patiño LH, Castañeda S, Rincón CA, et al. Phylogenomic evidence of reinfection and persistence of SARS-CoV-2: first report from Colombia. Vaccines. 2021; 9(3): 282.

17. Yurkovetskiy L, Wang X, Pascal KE, Tomkins-Tinch C, Nyalile TP, Wang Y, et al. Structural and functional analysis of the D614G SARS-CoV-2 spike protein variant. Cell. 2020; 183(3): 739-51.

18. Bianchi M, Borsetti A, Ciccozzi M, Pascarella S. SARS-Cov-2 ORF3a: mutability and function. Int J Biol Macromol. 2021; 170: 820-6.

19. Oulas A, Zanti M, Tomazou M, Zachariou M, Minadakis G, Bourdakou MM, et al. Generalized linear models provide a measure of virulence for specific mutations in SARS-CoV-2 strains. PLoS One. 2021; 16(1): 1-17.

20. INS - Instituto Nacional De Salud. COVID-19 en Colombia. 2022. Available from: https://www.ins.gov.co/Noticias/Paginas/coronavirus-filtro.aspx.

21. Garay E, Serrano-Coll H, Rivero R, Gastelbondo B, Faccini-Martínez Á, Berrocal J, et al. SARS-CoV-2 in eight municipalities of the Colombian tropics: high immunity, clinical and sociodemographic outcomes. Trans R Soc Trop Med Hyg. 2021; 1-9.

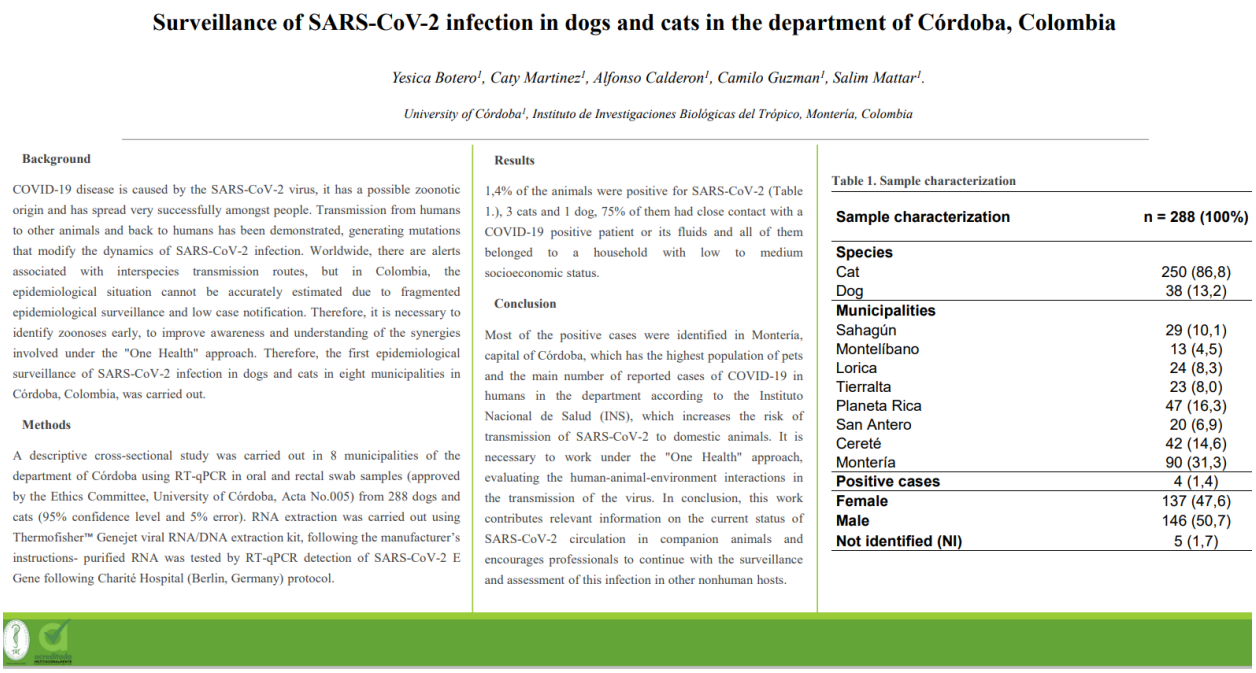
22. Prince T, Smith SL, Radford AD, Solomon T, Hughes GL, Patterson EI. SARS-CoV-2 infections in animals: reservoirs for reverse zoonosis and models for study. Viruses. 2021; 13(3): 494.

23. Suarez DL, Pantin-Jackwood MJ, Swayne DE, Lee SA, DeBlois SM, Spackman E. Lack of susceptibility to SARS-CoV-2 and MERS-CoV in Poultry. Emerg Infect Dis. 2020; 26(12): 3074-6.

24. Marbán E, Mattar S, González T. Las zoonosis reemergentes bajo el enfoque de "Una salud". Rev MVZ Cordoba. 2019; 24(3): 7280-4.

CAPÍTULO 2

En cumplimiento al primer y último objetivo, se presentó a modo de poster y video con presentación PowerPoint el resumen del presente trabajo titulado “Surveillance of SARS-CoV-2 infection in dogs and cats in the department of Córdoba, Colombia” en el evento internacional “COVID-19, Influenza and RSV: Surveillance-Informed Prevention and Treatment ISIRV” en octubre 19,20 y 21 de 2021. Se transmitió el conocimiento generado a través de medios online como redes sociales y página web **(Anexo 1.) (Anexo 2.) (Anexo 3.)**



Surveillance of SARS-CoV-2 infection in dogs and cats in the department of Córdoba, Colombia

Yesica Botero[1], Caty Martinez[1], Alfonso Calderon[1], Camilo Guzman[1], Salim Mattar[1].

University of Córdoba[1], Instituto de Investigaciones Biológicas del Trópico, Montería, Colombia

Background

COVID-19 disease is caused by the SARS-CoV-2 virus, it has a possible zoonotic origin and has spread very successfully amongst people. Transmission from humans to other animals and back to humans has been demonstrated, generating mutations that modify the dynamics of SARS-CoV-2 infection. Worldwide, there are alerts associated with interspecies transmission routes, but in Colombia, the epidemiological situation cannot be accurately estimated due to fragmented epidemiological surveillance and low case notification. Therefore, it is necessary to identify zoonoses early, to improve awareness and understanding of the synergies involved under the "One Health" approach. Therefore, the first epidemiological surveillance of SARS-CoV-2 infection in dogs and cats in eight municipalities in Córdoba, Colombia, was carried out.

Methods

A descriptive cross-sectional study was carried out in 8 municipalities of the department of Córdoba using RT-qPCR in oral and rectal swab samples (approved by the Ethics Committee, University of Córdoba, Acta No.005) from 288 dogs and cats (95% confidence level and 5% error). RNA extraction was carried out using Thermofisher™ Genejet viral RNA/DNA extraction kit, following the manufacturer's instructions- purified RNA was tested by RT-qPCR detection of SARS-CoV-2 E Gene following Charité Hospital (Berlin, Germany) protocol.

Results

1,4% of the animals were positive for SARS-CoV-2 (Table 1.), 3 cats and 1 dog, 75% of them had close contact with a COVID-19 positive patient or its fluids and all of them belonged to a household with low to medium socioeconomic status.

Conclusion

Most of the positive cases were identified in Montería, capital of Córdoba, which has the highest population of pets and the main number of reported cases of COVID-19 in humans in the department according to the Instituto Nacional de Salud (INS), which increases the risk of transmission of SARS-CoV-2 to domestic animals. It is necessary to work under the "One Health" approach, evaluating the human-animal-environment interactions in the transmission of the virus. In conclusion, this work contributes relevant information on the current status of SARS-CoV-2 circulation in companion animals and encourages professionals to continue with the surveillance and assessment of this infection in other nonhuman hosts.

Table 1. Sample characterization

Sample characterization	n = 288 (100%)
Species	
Cat	250 (86,8)
Dog	38 (13,2)
Municipalities	
Sahagún	29 (10,1)
Montelíbano	13 (4,5)
Lorica	24 (8,3)
Tierralta	23 (8,0)
Planeta Rica	47 (16,3)
San Antero	20 (6,9)
Cereté	42 (14,6)
Montería	90 (31,3)
Positive cases	4 (1,4)
Female	137 (47,6)
Male	146 (50,7)
Not identified (NI)	5 (1,7)

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ATTENDANCE CERTIFICATE

This is to certify that:

Yesica Botero

Attended the virtual conference 'COVID-19, Influenza and RSV: Surveillance-Informed Prevention and Treatment', 19th-21st October 2021 and had a poster presentation (#32) entitled 'Surveillance of SARS-CoV-2 infection in dogs and cats in the department of Córdoba, Colombia'

Best wishes

Frederick G. Hayden, M.D.
Scientific Committee Co-Chair
Professor Emeritus
University of Virginia
Charlottesville, VA
USA

Dr Wenqing Zhang
Scientific Committee Co-Chair
Head, Global Influenza Programme
World Health Organization
Geneva
Switzerland

isirv Antiviral Group - website www.isirv.org
ISIRV Ltd Registered Address: 54a Castle Road, Isleworth, Middlesex, TW7 6QS, England

CAPÍTULO 3

En cumplimiento al primer y segundo objetivo del presente trabajo se presentó el artículo titulado “Human-to-dog Transmission of SARS-CoV-2 Lota Variant: Should COVID-19 Patients Avoid Close Contact with their Pets During Illness?” en la revista Q1 Scientific Reports con DOI: https://doi.org/10.21203/rs.3.rs-821033/v1.

OPEN

Human-to-dog transmission of SARS-CoV-2, Colombia

Ricardo Rivero[1], Evelin Garay[1], Yesica Botero[1], Héctor Serrano-Coll[1], Bertha Gastelbondo[1], Marina Muñoz[2], Nathalia Ballesteros[2], Sergio Castañeda[2], Luz Helena Patiño[2], Juan David Ramirez[2], Alfonso Calderon[1], Camilo Guzmán[1], Caty Martinez-Bravo[1], Ader Aleman[1], Germán Arrieta[1,3] & Salim Mattar[1,3]✉

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of the current COVID-19 pandemic, has evolved to have a wide range of hosts, including non-human primates, wild and domestic animals. The ACE2 protein has a high level of conservation and is the common receptor invertebrate species for a viral infection to occur; this receptor could give rise to anthroponotic events. This article describes the first event of symptomatic transmission in Latin America from a human to a dog by the B.1.625 lineage of SARS-CoV-2. We found 21 shared mutations in the complete genomes of viral sequences from owners and dogs. Further phylogenetic and molecular analysis showed that 100% co-localization of the clade helps to understand human-animal transmission. Prediction of the Spike protein structure of the sequenced virus and docking analyzes showed that the E484K mutation in the receptor-binding domain (RBD) could contribute to the viral affinity of dACE2. Therefore, close contact between SARS-CoV-2-infected humans and pets should be avoided to prevent the emergence of novel mutations of public health importance from anthroponotic events.

Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of the current COVID-19 pandemic is a novel virus belonging to the *Betacoronavirus* genus and it is genetically closer to bat coronaviruses than human SARS. For the reasons mentioned above, it is known as a viral zoonosis[1,2]. Research on transmission mechanisms of the viruses focuses on person-to-person contact. However, domestic animals' susceptibility to this virus is still uncertain[3]. To date, 11 complete SARS-CoV-2 genomes isolated from dogs have been reported in the Global Initiative on Sharing All Influenza Data (GISAID), from oropharyngeal samples[4]. The mechanism for viral infections depends on the binding between SARS-CoV-2 S Protein Receptor Binding Domain (RDB) and angiotensin-converting enzyme 2 (ACE2) receptor, which is crucial for infection since it allows the internalization of the virion into host cells[5]. Recently, several SARS-CoV-2 variants have emerged with an enhanced affinity towards human ACE2[6]. Considering that ACE2 receptors are present in several animal species, interspecies infections could arise from human-to-animal contact. However, the efficacy of the cellular union depends on the affinity of the viral RBD towards the host's receptors[7,8]. Recently, several variants of SARS-CoV-2 have emerged with an augmented infectious capacity and neutralization-escape ability, these variants carry mutations in the *spike* protein such as N501Y, E484K, and K417T which have been described to have a relationship with higher transmissibility and resistance toward natural-induced and vaccine-elicited neutralizing antibodies[9–11]. In January 2021, a novel lineage identified as B.1.625 was reported in 5.8% of sequenced genomes in Colombia during the first trimester. Due to its rapid augment in prevalence and the identification of characteristic mutations in *Spike's* N-terminal Domain (NTD), Receptor Binding Domain (RBD) and S1/S2 accounting for increased transmissibility, drug-resistance and antibody escape it was a rapidly growing lineage that circulated through America and Europe. The B.1.625 lineage is now circulating in 10 countries, including Colombia, and continues to pose a threat to public health and vaccine efficacy[12–14]. It is still unknown if the emergence of novel variants of concern and interest with augmented transmissibility account for enhanced infectivity of non-human hosts. Research of a more significant scale under the "One Health" approach is needed to assess the feasibility of direct human-to-animal transmission of SARS-CoV-2 in domestic environments to understand better the dynamics of the viral infections and their risk towards other species, including humans. This research performs viral genome analysis through next-generation sequencing of two SARS-CoV-2 clinical isolates from a dog and its owner. Given

[1]Instituto de Investigaciones Biológicas del Trópico (IIBT), Facultad de Medicina Veterinaria y Zootecnia, Universidad de Córdoba, Montería, Colombia. [2]Centro de Investigaciones en Microbiología y Biotecnología-UR (CIMBIUR), Facultad de Ciencias Naturales, Universidad del Rosario, Bogotá, Colombia. [3]Clínica Salud Social, Sincelejo, Sucre, Colombia. ✉email: smattar@correo.unicordoba.edu.co

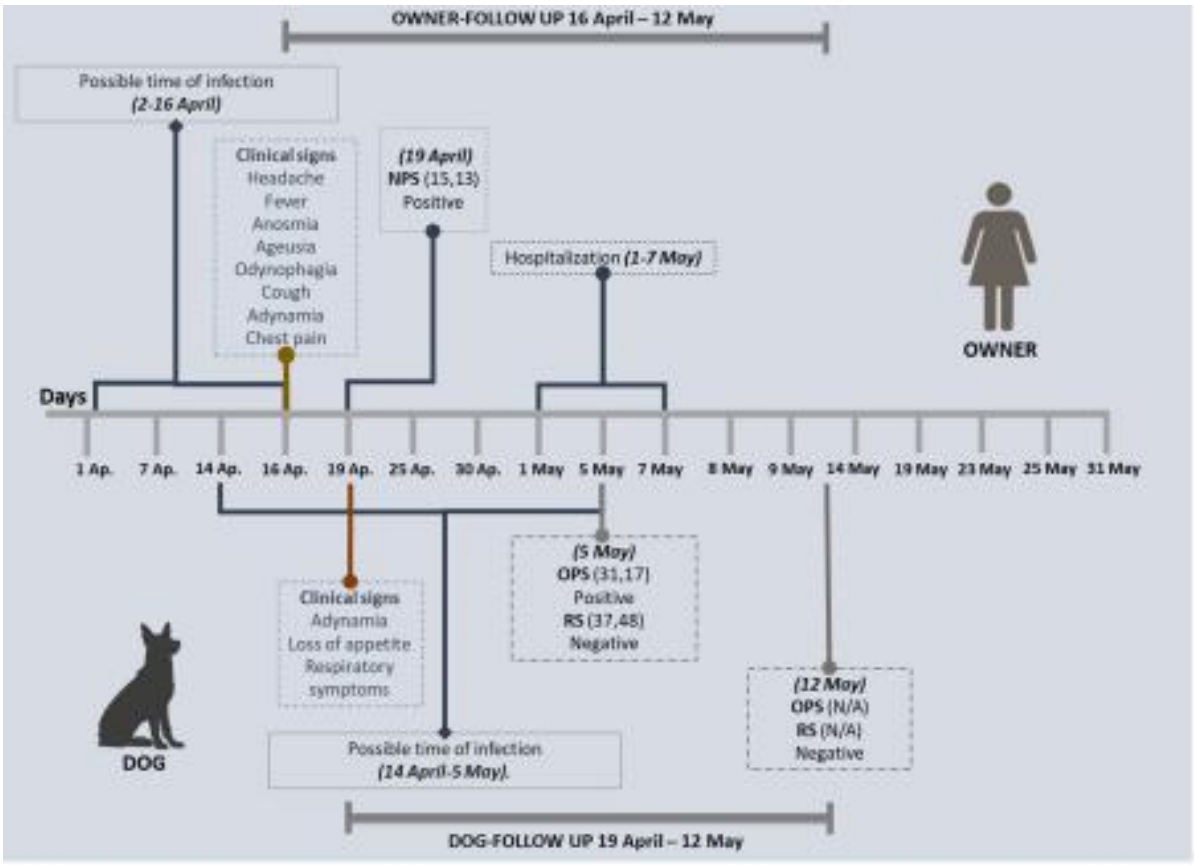

Figure 1. Timeline of symptom diagnosis, molecular testing, and follow-up.

the relevance of this issue, this research aimed to perform a molecular, phylogenetic, and molecular docking approach to a case of SARS-CoV-2 human-to-dog transmission in Colombia.

Results

Clinical case description of the infected canine. A 54-year-old female presenting moderate clinical manifestations of COVID-19, such as fever, cough, headache, dyspnea, chest pain, and compromise of the lung parenchyma, tested positive for SARS-CoV-2 by RT-qPCR on April 19th, 2021, with a Ct = 15.13. Her two years old German shepherd dog presented sneezing, cough, hyaline rhinorrhea, diarrhea, vomiting, adynamia, and lack of appetite for three days after having frequent close contact with its unvaccinated owner, which was in knowledge of the research being done by Universidad de Cordoba and reached out to researchers for pet's sample collection. RT-qPCR was conducted from oropharyngeal and rectal swab samples collected from the dog, resulting in a positive SARS-CoV-2 diagnostic by RT-qPCR (Ct = 31.36) on May 5th, 2021. A week later, a follow-up RT-qPCR test was conducted with a negative result in oropharyngeal and rectal swab samples. The animal did not show any other sign of illness after the negative result (Fig. 1). It is essential to mention that the dog had a strict indoor lifestyle and does not live with other pets. From the 290 domestic animals evaluated in this research study, 87.6% were felines and 12.4% were canines. RT-qPCR detected SARS-CoV-2 in a cat (EPI_ISL2339859.2) and a dog for total active infection rate of 0.69%.

Molecular and phylogenetic characteristics of viral genomes. Two full-genome sequences labeled anonymously as U118 (EPI_ISL_8422792) and U117 (EPI_ISL_8422346) were generated from RNA samples of an infected dog and its owner. The resulting SARS-CoV-2 genomes were compared with the reference NC_045512.2-Wuhan-Hu-1, lineage assignment in Pango Lineages[15] identified both sequences as part of B.1.625 lineage, which is located within the 20A clade and more specifically within the E484K sub-clade[16] (Fig. 2). These genomes showed a 91% sequence similarity and shared significant mutations and deletions in M, N, ORF1a, ORF1b, ORF3a and S. B.1.625's lineage defining T95I mutation was found in the viral spike protein among others such as E484K, D614G, N440K, as well as ΔH69-V70 and ΔY144 deletions (Table 1), Phylogenetic analysis by maximum-likelihood co-located both sequences within a sub-clade with a support of 100 alongside Colombian B.1.625 sequences (Fig. 2), and Twenty-one out of 25 mutations found in U118 were also found within U117 viral genome, QC of obtained sequences confirmed a %Ns of 9.04 in dog sample, including a stretch of Ns

located in S gene (21,147–21,386) (see Supplementary Data S1). Thus, it was confirming the transmission event between the human and its dog.

Predicted structure of the isolated SARS-CoV-2 Spike protein and molecular docking of RBD and dACE2. Predicted 3D structures were generated from the sequence U118 for N-terminal domain, Receptor Binding Domain and S2 of the B.1625 S protein (Fig. 3). Docking analyses for B.1.625 RBD-dACE2 and wtRBD-dACE2 showed a standard free binding energy (ΔG°) of −295.58 and −264.11 kJ mol^{-1}, respectively, which suggests a higher affinity of B.1.625's RBD towards dACE2 compared to wild-type SARS-CoV-2 RBD. Also, electrostatic potential mapping evidenced that aminoacid substitutions found in B.1.625's RBD predicted structure accounted for more positively charged residues when compared to wild-type protein, which could play a role in augmenting viral protein's affinity towards the receptor (Fig. 4). Molecular docking analysis between B.1.625 RBD and dACE2, identified 27 interface residues within 5 Å distance including N501, K484 and K417 (Fig. 5).

Discussion

Our results report the first case of human-to-dog transmission of SARS-CoV-2 in Latin America with successful isolation and sequencing of both viral full-genomes, and show concordance with previously reported studies on SARS-CoV-2 infection in dogs that had prolonged close contact with their owners[3,17]. A seroprevalence study carried out in Croatia found a 7.56% seropositivity in 172 randomly selected dogs living with healthcare, laboratory and veterinary personnel[17]. On the other hand, studies done in Spain and Italy could not obtain positive RT-qPCR from dog samples exhibiting pulmonary complications, but found a seroprevalence of 25% from dogs living in COVID-19(+) households, this indicates their susceptibility to SARS-CoV-2 infection and its relationship to close-contact with humans rather than dog-to-dog[7,18].

An experimental infection carried by Bosco-Lauth et al.[8] in cats and dogs that were inoculated with 3.0e5 and 1.45e5 PFU intranasally evidenced viral shedding occurring up to 5 days post-infection (DPI) in cats and moderate ulcerative, suppurative lympho- plasmocytic rhinitis in the nasal turbinates along with mild lymphoplasmacytic tracheitis but showed no signs of clinical disease, no weight-loss and body temperature < 39.5 °C, viral isolation was accomplished from trachea, nasal turbinates and esophagus of necropsied cats on day 5 post-infection. Contrastingly, no viral shedding nor clinical signs of disease was found in the experimental group of infected dogs with a seroconversion against RBD at 14 DPI[8]. In our study, although we did not perform viral isolation from samples, the upper respiratory tract clinical signs of disease observed in the infected dog could suggest active viral replication in lung tissue as well as in intestinal tract evidenced by the appearance of gastrointestinal signs such as vomiting, diarrhea, adynamia and lack of appetite.

The molecular and phylogenetic analyses of the obtained sequences support our hypothesis of a transmission event between the dog and its human owner, with both genomes being located within the same clade with a branch support of 100 and a total of 21 mutations shared between both sequences across isolated viral genomes. It is remarkable that these infections were due a Colombian SARS-CoV-2 lineage (B.1.625) which carries several mutations in the *spike* protein that confers augmented transmissibility and resistance to neutralization by naturally acquired and vaccine-elicited antibodies such as E484K mutation, which has been related to attenuation of anti-RBD neutralization[19] and N440K has been observed to reduce viral susceptibility (up to 28-fold) to monoclonal antibody-based antiviral treatment[14]. This mutations reduces complementarity and electrostatic affinity between neutralizing antibodies and RBD, which could promote an enhanced viral immune evasion in both humans and animals, as well as an increase in reinfection cases and reduction the efficacy of anti-SARS-CoV-2 vaccines[12,20,21].

Other spike mutations found in the obtained sequences such as N440K and D614G have been observed to reduce the efficacy of monoclonal antibody-based antiviral treatment up to 28-fold and augment the viral infectivity[14,22]. Furthermore, it is essential to emphasize that mutation D164G confers increased transmissibility to SARS-CoV-2 by stabilizing Spike protein, this prevents the S1 and S2 segments to be cleaved before RBD interacts with ACE2 receptor[23]. This could be associated with the greater affinity observed by Zhang et al.[24] towards dog ACE2 (dACE2) of variants carrying D614G mutation when compared to Wu-1 SARS-CoV-2. Therefore, mutation D614G could play a key role for anthropozoonotic transmission of SARS-CoV-2[24].

On the other hand, in vitro studies have reported that N501Y mutation found in several variants such as Alpha (B.1.1.7), Gamma (P.1) and Delta (B.1.617.2) enhances the affinity of RBD towards dACE2, accounting for a threefold increase in affinity (K_D = 37.1). Hence, wild-type RBD (K_D = 123 nM), augmented affinity and higher viral loads found in lineages carrying D614G mutation that was found in the present study, and it could cause an increase in human-to-animal transmission[22,24].

The virological landscape in Colombia during the time of the study was characterized by the co-circulation of several lineages including Alpha, Gamma and Delta VOCs as well as Mu VOI which accounted for a percentage of 2.17, 12.8, 8.87 and 57.3 of all the reported sequenced between January and August of 2021, showing that the circulation of B.1.625 was very limited to the persistence and dominance of other lineages with enhanced transmissibility and immune escape[25–28].

Zooanthroponosis has occurred in a mink farm in Denmark. this led to the culling of more than 17 million animals, and the emergence of a novel SARS-CoV-2 lineage with reduced susceptibility to antibody-neutralization (Mink cluster V)[6,29–31]. Due to the broad host range of SARS-CoV-2, self-isolation measures should be taken by humans to avoid the occurrence of zooanthroponosis events and public health risks which could lead to the emergence of novel lineages with new mutations of importance[32].

Our study shows in silico that these mutations could account for an increase in the risk of zooanthroponosis events. However, in vitro experiments should be carried out for determining the affinity of VOC and VOI RBD

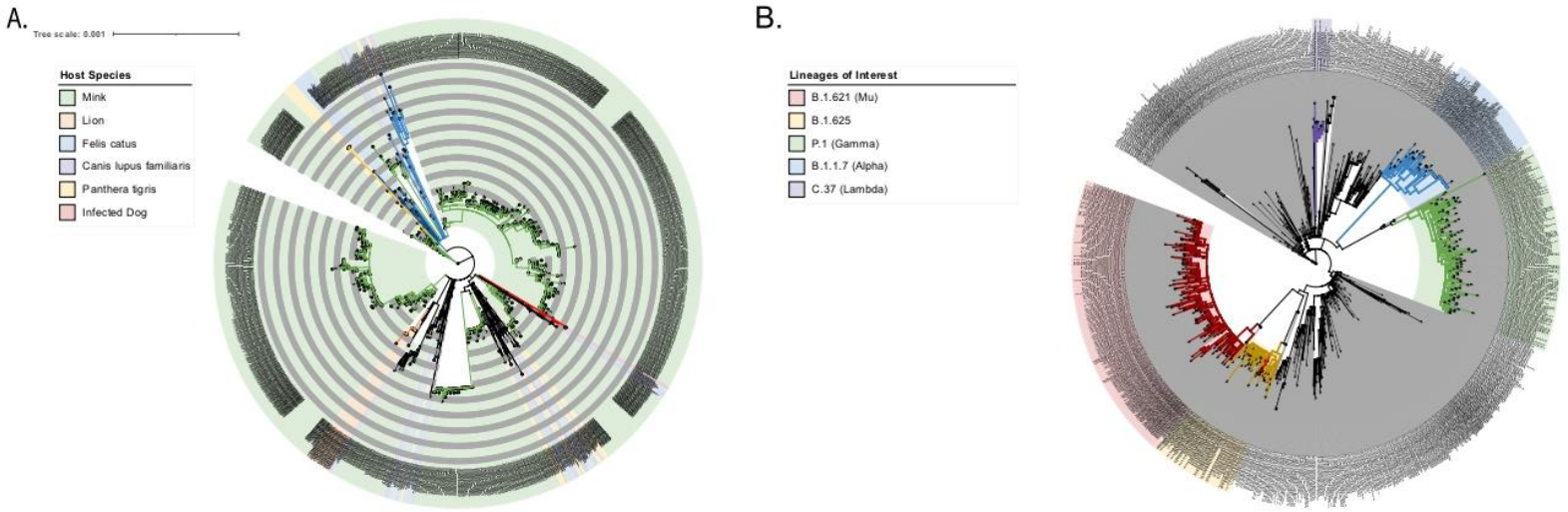

Figure 2. (**A**) Phylogenetic trees of sequences available in GISAID from minks, lions, tigers, cats, and dogs, U117 is highlighted in red. (**B**) Phylogenetic tree comprising Colombian sequences, VOC, and lineages of interest are annotated within the tree; U117 and human index case U118 are highlighted in red.

Host (species)	ID	Lineage	Mutations observed*
Canis lupus familiaris	U117	*B.1.625*	(n = 21)—**M** (I82T), **N** (M234I); **ORF1a** (V86F), **ORF1a** (L681F), **ORF1a** (K1319E), **ORF1a** (T1822I), **ORF1a** (P2046S), **ORF1a** (K3353R), **ORF1a** (P3504S), **ORF1a** (ΔS3675-F3677); **ORF1b** (K82R), **ORF1a** (P314L), **ORF1a** (T1076I); **ORF3a** (Q57H); **ORF8** (ΔK2); **S** (F157L), **S** (N440K), **S** (E484K), **S** (D614G), **S** (D950N), **S** (V1228L)
Homo sapiens sapiens	U118	*B.1.625*	(n = 25)—**M** (I82T); **N** (M234I); **ORF1a** (V86F), **ORF1a** (L681F), **ORF1a** (K1319E), **ORF1a** (T1822I), **ORF1a** (P2046S), **ORF1a** (G2509D), **ORF1a** (K3353R), **ORF1a** (P3504S), **ORF1a** (ΔS3675-F3677); **ORF1b** (K82R), **ORF1b** (P314L), **ORF1b** (T1076I); **ORF3a** (Q57H); **ORF7a** (I110V); **ORF8** (ΔK2); **S** (T95I), **S** (F157L), **S** (N440K), **S** (E484K), **S** (D614G), **S** (D950N), **S** (V1228L), **S** (ΔH69-V70), **S** (ΔY144)

Table 1. Details of the SARS-Cov-2 genomes sequenced. *In parentheses: amino acid substitutions/position aligned with reference NC_045512.2-Wuhan-Hu-1. *N* nucleoprotein, *M* membrane glycoprotein M, *ORF* open reading frame, *S* spike protein. Proteins with aminoacid substitutions are in [bold].

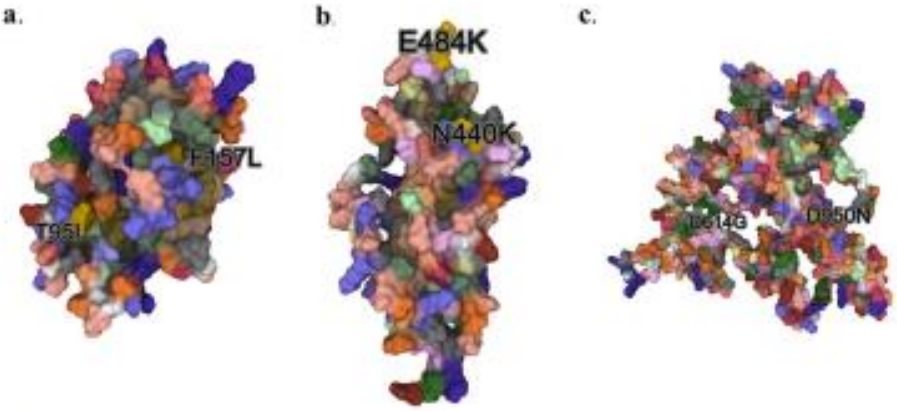

Figure 3. Predicted structure models of B.1.625 SARS-CoV-2 Spike Protein domains (**a**–**c**) N-terminal Domain, Receptor Binding Domain, and S1/S2 with mutations highlighted in yellow.

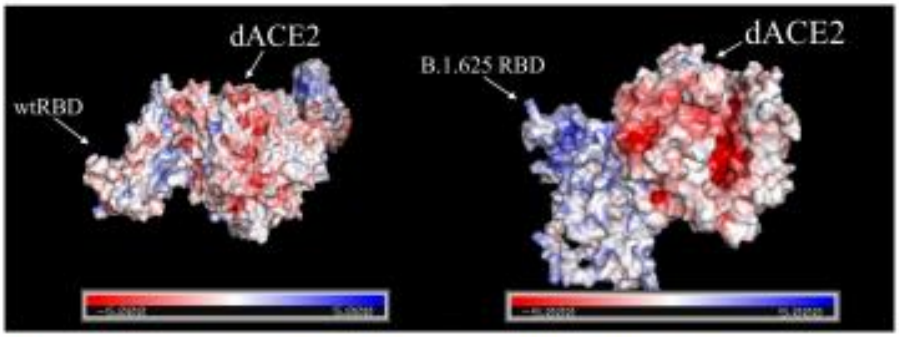

Figure 4. Electrostatic potential map of wtRBD-dACE2 and B.1.625 RBD-dACE2.

towards animal hosts to assess the impact of the emergence of these novel and more transmissible lineages in non-human hosts.

In conclusion, the present study was the first reported case of a human-to-dog transmission event in Latin America supported by the sequencing and molecular characterization of both viral genomes. The identification of the B.1.625 lineage as the causative agent of this infection adds to the discussion on the importance of self-isolation measures between infected humans and pets. Considering the emergence of novel and more transmissible variants of SARS-CoV-2 could have an enhanced capability of infecting non-human hosts. Epidemiological and molecular surveillance of zooanthroponosis in pets co-habiting with SARS-CoV-2 patients is mandatory since it could represent an animal mutations risk for public health.

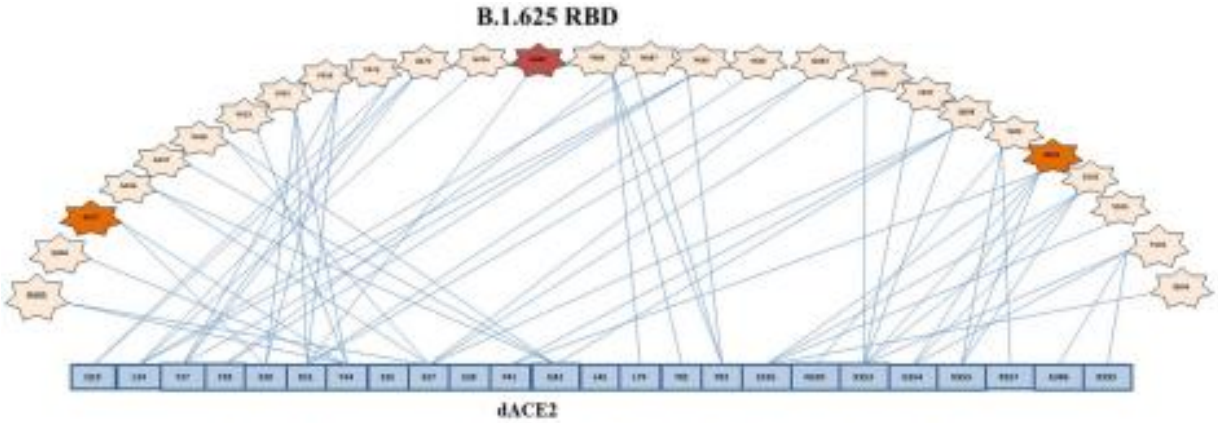

Figure 5. B.1.625 SARS-CoV-2-dACE2 interface residues. Mutation E484K is presented in red; residues 417 and 501 of importance in transmissibility enhancement are highlighted in orange.

Methods

Sample collection and detection of SARS-CoV-2. Two hundred ninety samples were collected from domestic animals in 8 municipalities of the Cordoba department located in the north-western Colombian Caribbean, informed consent was obtained from human participants for sample collection in this study. Oropharyngeal and rectal swabs were collected for felines and canines as well as nasopharyngeal samples for humans as a part of the genomic and epidemiologic surveillance of pets living in COVID-19(+) patients' households. Samples were conserved in a Viral Transport medium for SARS-CoV-2 detection by RT-qPCR. Briefly, RNA extraction was carried out using Thermofisher Genejet viral RNA/DNA extraction kit by following the manufacturer's instructions for swab samples. Then, purified RNA was tested by RT-qPCR detection of SARS-CoV-2 E Gene following Charité Berlin protocol[33]. Those animal samples with a Ct value ≤ 32 was then subjected to next-generation sequencing of the virus' complete genome. This study was approved by Universidad de Cordoba's Veterinary Medicine faculty and its Ethics Committee number 005 (May 26th, 2021). All methods were carried out in accordance with national and international guidelines, including Law 84 of 1989 of the Congress of the Republic of Colombia, national guidelines of animal protection, resolution 8430 of Colombia's Health Ministry and articles 87 and 88 of the 1989's Universal declaration of Animal Rights. Additionally, animals and humans were sampled in compliance of CDC's Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories and WHO Laboratory Biosafety Manual, ensuring human and animal wellbeing[34,35]. All methods were reported in accordance with the ARRIVE guidelines for the reporting of animal experiments[36].

Sequencing of RT-qPCR positive samples. Samples were subjected to whole-genome sequencing. Sequence libraries were prepared from RNA extracted from each nasopharyngeal/oropharyngeal swab per individual using the ARTIC Network protocol (https://artic.network/ncov-2019). Long-read Oxford Nanopore MinION sequencing was performed with the MinKNOW application (v1.5.5). Initially, raw Fast5 files were base called, and demultiplexed using Guppy; subsequently, reads were filtered by quality and length, eliminating possible chimeric and low-quality reads. Finally, genome assemblies were obtained following the MinION pipeline described in the ARTIC bioinformatics pipeline (https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html). Each assembly was typed based on the PANGOLIN nomenclature lineage allocator and SNPs identification was performed using the clade assignment, mutation calling and sequence quality checking tool, NextClade, which identifies differences between sequences and a reference sequence used by Nextstrain.

Phylogenetic analysis of the sequences. Briefly, two datasets were generated from virus sequences reported in GISAID. For humans, 1855 viruses have been reported from Colombia in GISAID, incomplete sequences (< 29,000 bp) and sequences with low coverage (> 5% Ns) were excluded from the analysis resulting in a final dataset of 1002 sequences including the dog and owner. On the other hand, another dataset was created with sequences of SARS-CoV-2 detected in *Canis lupus familiaris* (dog), *Felis catus* (cat), *Panthera leo* (lion), *Panthera tigris* (tiger) and *Neovison vison* (mink) reported in GISAID globally by following the selection criteria as mentioned above to evaluate phylogenetic between globally reported sequences from cohabiting animals. The two datasets composed of a total of 1002 and 1057 sequences were subjected to multiple sequence alignment (MSA) against reference sequence NC_04551 in MAFFT v 7.48[37] using "--addfragments"[37] functionality within the webserver. Then, alignments were manually edited in Bioedit v.7.2.5 in order to add sample collection date to FASTA accession IDs as defined by REGEX (\d\d\d\d-\d\d-\d\d). Maximum-likelihood phylogenetic trees were reconstructed for each dataset in IQtree v.2.1.3[38], ModelFinder[39] was run for the datasets to determine the best-fit substitution model, this resulted in GTR + F + I being selected for tree reconstruction, branch support was calculated using UFBoot2[40] within IQTree2. The consensus tree was visualized and tailored in FigTree v.1.4.4[41]

and rooted to NC_045512. Trees were exported in Newick format and then edited in iTOL[42] for the addition of annotation schemes.

Structural and molecular docking analysis of SARS-CoV-2 B.1.625 Spike protein. Predicted 3D models of *spike* protein domains, namely Amino-terminal Domain (NTD), Receptor Binding Domain (RBD), and S1/S2 were constructed in AlphaFold2[43] from the sequence of SARS-CoV-2 B.1.625 lineage retrieved from the infected human, multiple sequence alignment for AlphaFold2 was done in MMseqs2[44]. Then, molecular docking analysis was carried out in HDOCK for comparing the affinity of wild-type SARS-CoV-2 RBD (wtRBD) (Protein Database accession ID 6M0J:B)[45] and B.1.625 RBD towards both Human (hACE2) and dog (dACE2)[24] angiotensin converting enzyme 2 (6M0J:A and 7E3J:A, respectively). Generated models for each interaction were ranked according to Standard binding free energy ($\Delta G°$)[46]. Poisson-Boltzmann electrostatics visualizations were generated with PDB2PQR plugin within PyMOL[47].

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References

1. Reina, J. E. SARS-CoV-2, una nueva zoonosis pandémica que amenaza al mundo. *Vacunas* **21**, 17–22 (2020).
2. World Health Organization. WHO coronavirus (COVID-19) Dashboard. WHO coronavirus (COVID-19) dashboard with vaccination data. *Who* 1–5 (2021).
3. Sit, T. H. C. *et al.* Infection of dogs with SARS-CoV-2. *Nature* **586**, 776–778 (2020).
4. Elbe, S. & Buckland-Merrett, G. Data, disease and diplomacy: GISAID's innovative contribution to global health. *Glob. Challenges* **1**, 33–46 (2017).
5. Premkumar, L. *et al.* The receptor-binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. *Sci. Immunol.* **5**, 1–9 (2020).
6. Gobeil, S.M.-C. *et al.* Effect of natural mutations of SARS-CoV-2 on spike structure, conformation and antigenicity. *bioRxiv Prepr. Serv. Biol.* https://doi.org/10.1101/2021.03.11.435037 (2021).
7. Goumenou, M., Spandidos, D. A. & Tsatsakis, A. Possibility of transmission through dogs being a contributing factor to the extreme COVID-19 outbreak in North Italy. *Mol. Med. Rep.* **21**, 2293–2295 (2020).
8. Bosco-Lauth, A. M. *et al.* Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to reexposure in cats. *Proc. Natl. Acad. Sci. USA* **117**, 26382–26388 (2020).
9. Jangra, S. *et al.* SARS-CoV-2 spike E484K mutation reduces antibody neutralisation. *Lancet Microbe*. (2021).
10. Wibmer, C. K. *et al.* SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. *Nat. Med.* https://doi.org/10.1038/s41591-021-01285-x (2021).
11. Focosi, D. & Maggi, F. Neutralising antibody escape of SARS-CoV-2 spike protein: Risk assessment for antibody-based COVID-19 therapeutics and vaccines. *Rev. Med. Virol.* https://doi.org/10.1002/rmv.2231 (2021).
12. West, A. P., Barnes, C. O., Yang, Z. & Bjorkman, P. J. SARS-CoV-2 lineage B.1.526 emerging in the New York region detected by software utility created to query the spike mutational landscape. https://doi.org/10.1101/2021.02.14.431043.
13. Annavajhala, M. K. *et al.* A novel and expanding SARS-CoV-2 variant, B.1.526, identified in New York. https://doi.org/10.1101/2021.02.23.21252259.
14. Federation Drug American (FDA). Fact sheet for health care providers emergency use authorization of bamlanivimab and etesevimab. 1–36 (2020).
15. O'Toole, Á. *et al.* pangolin: Lineage assignment in an emerging pandemic as an epidemiological tool. in *prep*. https://doi.org/10.1093/ve/veab064/6315289. (2021).
16. Rambaut, A. *et al.* A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat. Microbiol.* **5**, 1403–1407 (2020).
17. Stevanović, V. *et al.* Seroprevalence of SARS-CoV-2 infection among pet animals in Croatia and potential public health impact. *Transbound. Emerg. Dis.* **00**, 1–7 (2020).
18. Perisé-Barrios, A. J. *et al.* Humoral responses to SARS-CoV-2 by healthy and sick dogs during the COVID-19 pandemic in Spain. *Vet. Res.* **52**, 1–11 (2021).
19. Jangra, S. *et al.* SARS-CoV-2 spike E484K mutation reduces antibody neutralisation. *Lancet Microbe* **2**, e283–e284 (2021).
20. Cele, S. *et al.* Escape of SARS-CoV-2 501YV2 from neutralization by convalescent plasma. *Nature* **593**, 142–146 (2021).
21. Andreano, E. *et al.* SARS-CoV-2 escape in vitro from a highly neutralizing COVID-19 convalescent plasma. *bioRxiv Prepr. Serv. Biol.* https://doi.org/10.1101/2020.12.28.424451 (2020).
22. Fernández, A. Structural impact of mutation D614G in SARS-CoV-2 spike protein: Enhanced infectivity and therapeutic opportunity. *ACS Med. Chem. Lett.* **11**, 1667–1670 (2020).
23. Zhang, L. *et al.* SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. *Nat. Commun.* **11**, 1–9 (2020).
24. Zhang, Z. *et al.* The molecular basis for SARS-CoV-2 binding to dog ACE2. *Nat. Commun.* **12**, 4195 (2021).
25. Naveca, F. G. *et al.* COVID-19 in Amazonas, Brazil, was driven by the persistence of endemic lineages and P.1 emergence. *Nat. Med.* **27**, 1230–1238 (2021).
26. Faria, N. R. *et al.* Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science (80-).* **372**, 815–821 (2021).
27. du Plessis, L. *et al.* Establishment and lineage dynamics of the SARS-CoV-2 epidemic in the UK. *Science (80-).* **371**, 708–712 (2021).
28. Early introductions and transmission of SARS-CoV-2 variant B.1.1.7 in the United States Graphical abstract. https://doi.org/10.1016/j.cell.2021.03.061.
29. Mallapaty, S. COVID mink analysis shows mutations are not dangerous—Yet. *Nature* **587**, 340–341 (2020).
30. Oude Munnink, B. B. *et al.* Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans.
31. World Health Organization. SARS-CoV-2 mink-associated variant strain—Denmark. https://www.who.int/emergencies/disease-outbreak-news/item/2020-DON301. (Accesed 31 July 2021)
32. Luan, J., Lu, Y., Jin, X. & Zhang, L. Spike protein recognition of mammalian ACE2 predicts the host range and an optimized ACE2 for SARS-CoV-2 infection. (2020). https://doi.org/10.1016/j.bbrc.2020.03.047.
33. Corman, V. *et al.* Diagnostic detection of 2019-nCoV by real-time RT-PCR. https://virologie-ccm.charite.de/en/ (2020). (Accesed 16 Apr 2021)

CAPÍTULO 4

En cumplimiento al tercer objetivo del presente trabajo se redactó el artículo titulado "Seroprevalencia de SARS-CoV-2 en perros y gatos de ocho municipios en una región del Caribe Colombiano" el cual será presentado a una revista Q2.

Seroprevalencia de SARS-CoV-2 en perros y gatos de ocho municipios en una región del Caribe Colombiano

Seroprevalence of SARS-CoV-2 in dogs and cats of eight municipalities in a region of the Colombian Caribbean

Yesica Botero[1], Caty Martinez[1], Alfonso Calderon[1], Héctor Serrano-Coll[2], Camilo Guzman[1], Salim Mattar[1]

[1] Universidad de Córdoba, Instituto de Investigaciones Biológicas del Trópico, Montería, Colombia

[2] Universidad CES, Instituto Colombiano de Medicina Tropical, Medellín, Colombia

Resumen

Introducción. Desde el inicio de la pandemia por SARS-CoV-2 uno de los grupos de hospederos que más preocupación ha causado es los animales domésticos, debido al alto contacto con personas quienes representan el mayor número de casos positivos en el mundo y su variable susceptibilidad a este virus. Es necesario realizar un tamizaje serológico de los anticuerpos específicos contra SARS-CoV-2 en gatos y perros para conocer la prevalencia de esta infección. Por lo tanto, se determinó la seroprevalencia de SARS-CoV-2 en perros y gatos de Córdoba, Colombia. **Metodología.** Se realizo una vigilancia serológica descriptiva de corte transversal. Entre septiembre 2020 a septiembre 2021, se tomaron proporcionalmente 279 muestras sanguíneas de perros y gatos de 8 municipios del departamento de Córdoba. Todas las muestras fueron procesadas utilizando el kit comercial ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA. **Resultados.** Se detectaron anticuerpos contra la nucleocápside de SARS-CoV-2 en 11,5% de las muestras. Montelíbano presentó una mayor seroprevalencia de 26,3% lo cual coincide con la alta seroprevalencia de casos humanos en el momento de la toma de muestra como fue reportado por otros autores. **Discusión.** Los resultados indican que la seroprevalencia de SARS-CoV-2 en animales puede estar directamente relacionada con la alta prevalencia en humanos, sin embargo, se deben considerar otros factores que puedan influir, como la dinámica de anticuerpos de cada especie contra este virus.

Palabras clave. Salud pública, Una salud, serología, estudios seroepidemiológicos

Abstract.

Introduction. Since the beginning of the SARS-CoV-2 pandemic, one of the host groups that has caused most concern is domestic animals, due to their high contact with people who represent the largest number of positive cases in the world and their varying susceptibility to this virus. Serological screening for SARS-CoV-2 specific antibodies in cats and dogs is necessary to know the prevalence of this infection. Therefore, the seroprevalence of SARS-CoV-2 was determined in dogs and cats in Córdoba, Colombia. **Methodology.** A descriptive cross-sectional serological surveillance was performed. Between September 2020 and September 2021, 279 blood samples were proportionally collected from dogs and cats from 8 municipalities of the department of Córdoba. All samples were processed using the commercial ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA kit. **Results.** Antibodies against the SARS-CoV-2 nucleocapsid were detected in 11.5% of the samples. Montelibano presented a higher seroprevalence of 26.3% which was consistent with the high seroprevalence of human cases when sampling as reported by other authors. **Discussion.** The results indicate that the seroprevalence of SARS-CoV-2 in animals may be directly related to the high prevalence in humans; however, other factors should be considered that may influence, such as the dynamics of antibodies of each species against this virus.

Keywords. Public health, One Health, serology, seroepidemiologic studies

Introducción.

Desde el inicio de la pandemia por SARS-CoV-2, virus causante del COVID-19 en humanos, se ha demostrado a través de modelos predictivos, infecciones naturales e in-situ el amplio rango de hospederos animales que puede abarcar este coronavirus dadas las condiciones y circunstancias adecuadas(1–3).

Uno de los grupos de hospederos que más preocupación ha causado es los animales domésticos, debido al alto contacto con personas quienes representan el mayor número de casos positivos en el mundo (2). Entre ellos se encuentran los gatos y perros considerados susceptibles debido a la afinidad del SARS-CoV-2 hacia el receptor ACE2 (angiotensin-coverting enzyme 2) (2,4–8). Esto ha causado una alta demanda de vigilancia epidemiológica en estos animales para la detección temprana de zoonosis inversa o spillback de este virus en poblaciones de mascotas, lo cual podría llevar a nuevos eventos de mutación con adaptación viral, surgimiento de nuevas variantes de interés, aumento de patogenicidad, evasión inmune y/o establecimiento de nuevos reservorios animales (7,9).

Aún no está claro el papel que juegan las mascotas en la propagación del virus, sin embargo, múltiples eventos de infección alrededor del mundo ponen en duda sobre el rol de estos animales en la transmisión del SARS-CoV-2 (7). Una de las herramientas más utilizadas para la detección de la infección en humanos es el ensayo molecular de PCR en tiempo real teniendo en cuenta la presencia de síntomas compatibles con COVID-19. Muchas veces los síntomas que presentan perros y gatos pasan desapercibidos y los ácidos nucleicos virales son detectables por un reducido periodo de tiempo (1).

Es necesario realizar un tamizaje serológico de los anticuerpos específicos contra SARS-CoV-2 en gatos y perros para conocer la prevalencia de esta infección en cada departamento del país. Por lo tanto, se tomaron de forma aleatoria y puntual muestras sanguíneas de perros y gatos con o sin contacto conocido con personas con COVID-19 las cuales se testearon utilizando un kit de ELISA comercial para determinar la tasa de infección de SARS-CoV-2 en perros y gatos del departamento de Córdoba entre septiembre 2020-2021.

Metodología.

Con la aprobación del comité de ética del Instituto de Investigaciones Biológicas del Trópico con No. 0410-2020 y del comité de ética de la facultad de Medicina Veterinaria y Zootecnia de la Universidad de Córdoba Acta No. 005 26 (May 2021). Y con consentimiento informado por parte de los tutores responsables de las mascotas incluidas en el estudio.

Entre septiembre 2020 a septiembre 2021, se tomaron de forma aleatoria y puntual proporcionalmente a la población estimada de mascotas 279 muestras sanguíneas

de perros y gatos con o sin contacto conocido con personas con COVID-19 en 8 municipios de un departamento de la Región Caribe colombiana que luego fueron procesadas con centrifuga para obtener suero sanguíneo y ser conservadas hasta su procesamiento. Todas las muestras fueron procesadas utilizando el kit comercial ID Screen® SARS-CoV-2 Double Antigen Multi-species ELISA para la detección de anticuerpos dirigidos contra la nucleocápside del SARS-CoV-2 en suero animal siguiendo las instrucciones del fabricante y realizando la lectura utilizando lector de placas CLARIOstar ^{Plus} BMG LABTECH. Se realizaron ninguna, una o dos replicas acorde a la cantidad de muestra disponible. Los valores DO≥0.350 son considerados positivos acorde a las instrucciones de manufactura del kit.

Se hizo seguimiento serológico de un perro y un gato que habían sido PCR positivas para SARS-CoV-2. Se les tomó una muestra el mismo día que se obtuvo el resultado PCR positivo y se hizo seguimiento cada uno o dos meses hasta obtener una muestra positiva.

Plan de análisis.

Los datos se tabularon en una base de datos de Excel y se analizaron utilizando el paquete estadístico Statistical Package for the Social Sciences versión 27 (IBM, Armonk, NY, USA) el cual se encuentra licenciado para el Instituto de Investigaciones Biológicas del Trópico (IIBT). El análisis univariado para las variables cualitativas se realizó a través del cálculo de frecuencias absolutas y relativas y para las variables cuantitativas se calcularon medidas de tendencia central (media y mediana). La normalidad de las variables se evaluó utilizando el Test Kolmogorov-Smirnov.

El análisis bivariado para las variables de naturaleza cualitativa se hizo a través del test de Chi-cuadrado de Pearson. Para la comparación de variables cualitativas politómicas con cuantitativas se llevó a cabo la prueba de Kruskal-Wallis y una comparación por parejas para determinar la significancia estadística entre los grupos evaluados.

Para todos los análisis el valor de significancia se consideró P<0,05. Además, se determinó para algunas variables el riesgo a través de la determinación del Odds Ratio (OR) con su respectivo intervalo de confianza 95%.

Resultados.

Características morfodemográficas.

Un total de 279 muestras fueron evaluados en Córdoba. 51,3% eran machos, 69,9% eran gatos, 46,9% de los animales positivos eran juveniles y cachorros (edad < 1 año) y 36% vivían en Montería. Se observo una seropositividad de anticuerpos totales contra la nucleocápside de SARS-CoV-2 de 11,5% **(Tabla 1.)**

Seropositividad y área geográfica.

Al comparar la seropositividad contra SARS-CoV-2 se observó una mayor seropositividad en el municipio de Montelíbano (26,3%), seguido del municipio de Cereté (21,6%). Por otra parte, se comparó la cinética de anticuerpos totales contra SARS-CoV-2 evidenciándose una diferencia estadísticamente significativa entre los municipios evaluados (P valor: 0,005) y además se realizó una comparación por parejas evidenciándose diferencias entre los municipios de Montelíbano-Montería, Montelíbano-Cereté y Montería-Sahagún (P valor:< 0,05) **(Figura 1.).**

Seropositividad para SARS-CoV-2 en relación con el rango etario.

Al comparar estas dos variables no se observaron diferencias entre los diferentes rangos etarios evaluados en los animales muestreados con respecto a los datos de seropositividad contra SARS-CoV-2 (P: 0,88) **(Tabla 2.).**

Seropositividad para SARS-CoV-2 asociado al sexo.

No se observó una diferencia estadística significativa entre la seropositividad en machos (65,6%) y hembras (34,4%) **(Tabla 3.).**

Seropositividad para SARS-CoV-2 con respecto a haber tenido contacto cercano con personas con COVID-19.

Al relacionar la positividad serológica en los animales evaluados en relación con haber tenido contacto estrecho con un individuo con COVID-19 no evidenciamos diferencia estadísticamente significativa al comparar estas dos variables (P>0,05). Además, no pudimos evidenciar que el contacto con una persona con COVID-19 se comportara como un factor de riesgo para adquirir esta infección (Tabla 4.)

Seguimiento.

Se obtuvo una muestra positiva de un perro y gato a los tres y cuatro meses respectivamente después de haberse detectado infección activa de SARS-CoV-2.

Discusión.

Se evidenció una seropositividad de 11,5% de anticuerpos totales similar a la descrita en un estudio en Estados Unidos, que mostró una seroprevalencia de anticuerpos contra la nucleocápside (N) de SARS-CoV-2 de 11%-12% en perros y gatos de Minnesota durante el primer semestre de 2020 (10). En Wuhan (China) tras el brote de COVID-19 se describió una seroprevalencia del 15% de IgG de SARS-CoV-2 RBD en gatos (11). En Italia, se encontró que el 3,3% de los perros y el 5,8% de los gatos tenían anticuerpos medibles contra SARS-CoV-2 (12).

Nuestros resultados indican que 32 perros y gatos en el departamento de Córdoba han estado expuestos a SARS-CoV-2 y han generado anticuerpos totales contra la N de este virus. La seroprevalencia en perros (19%) fue alta y mayor a la evidenciada en gatos (8,2%) **(Tabla 1.)** contrario a la reportado por otros autores (10–13).

Casi la mitad de las muestras seropositivas provinieron de perros y gatos con contacto directo confirmado con personas COVID-19 positivas, sin embargo, en el presente estudio no se comportó como un factor de riesgo (OR 1,4) para adquirir la infección por SARS-CoV-2 **(Tabla 4.)**.

Montelíbano (Córdoba), presentó la mayor seroprevalencia entre los municipios evaluados **(Figura 1.)** y la cinética de anticuerpos tuvo una diferencia estadísticamente significativa en relación a los demás municipios. Esto coincide con la alta seroprevalencia de casos humanos reportado en Córdoba, alrededor de las mismas fechas y zonas geográficas en que se tomaron las muestras del presente estudio, donde describen que Montelíbano presentó la mayor seropositividad (52,6%) (14).

Los resultados indican que la seroprevalencia de SARS-CoV-2 en animales puede estar directamente relacionada con la alta prevalencia de COVID-19 en humanos (**Figura 1.)**, sin embargo, se deben considerar otros factores que puedan influir, como la dinámica de anticuerpos de cada especie contra este virus. Ya que al realizar seguimiento a un perro y un gato, se evidencio una tardía seroconversión a los tres y cuatro meses respectivamente a comparación con lo reportado por otros autores en donde se evidencian anticuerpos tan temprano como 7 días y hasta 42 días post infección (1,4,6).

En Colombia no se cuenta con una Política Pública sobre Tenencia Responsable de Animales de Compañía que este acorde con la normatividad internacional relacionada con la protección y bienestar animal, por tanto, la regulación de una tenencia responsable es baja y predominan en algunas regiones del país los hábitos callejeros de las mascotas. Esto incrementa el riesgo de un posible evento de contagio fuera del hogar de donde proviene la mascota.

En conclusión, nuestro estudio proporcionó evidencia serológica de la infección por SARS-CoV-2 en mascotas de una región del Caribe colombiano, y se describió brevemente la dinámica de anticuerpos contra SARS-CoV-2 observado en un perro y un gato. Se hace necesario incrementar la vigilancia epidemiológica del virus SARS-CoV-2 en otros hospederos animales no humanos susceptibles para investigar la ruta de transmisión humano-animal-humano y llevar a cabo estudios acerca de la dinámica de los anticuerpos contra este virus en animales. Además, se recomienda no descuidar las medidas de bioseguridad, aislamiento y autocuidado en caso de presentar COVID-19. Finalmente, se sugiere una tenencia responsable y fomento del bienestar animal y estilo de vida *Indoor* de las mascotas.

	n=279 (100%)	n Positivos (%) 32 (11,5)
Especie		
Gato (*Felis catus)*	195 (69,9)	16 (8,2)
Perro (*Canis familiaris lupus)*	84 (30,1)	16 (19)
Edad		
≤ 1año	135 (48,4)	15 (46,9)
> 1 año	114 (40,9)	14 (43,7)
Desconocido	30 (10,7)	3 (9,4)
Ciudad		
Sahagún	37 (13,3)	2 (5,4)
Montelíbano	19 (6,8)	5 (26,3)
Lorica	30 (10,8)	6 (20,0)
Tierralta	20 (7,2)	0 (0)
Planeta Rica	26 (9,3)	2 (7,7)
Montería	101 (36,2)	9 (8,9)
San Antero	9 (3,2)	0 (0)
Cereté	37 (13,3)	8 (21,6)

Tabla 1. Características de los animales domésticos evaluados por ELISA.

Rango etario	Serología		Valor P
	Positivo (%)	Negativo (%)	
<12 meses	15 (11,1)	120 (88,9)	0,88
13-36 meses	7 (12,5)	49 (87,5)	
37-72 meses	3 (8,8)	31 (81,3)	
>73 meses	4 (16,7)	20 (83,3)	

Tabla 2. Comparación entre el rango etario con respecto a la seropositividad.

Sexo	Serología		Valor P
	Positivo (%)	Negativo (%)	
Macho	21 (14,7)	122 (85,3)	
Hembra	11 (8,3)	122 (91,7)	0,2
Desconocido	0	3 (100)	

Tabla 3. Comparación entre el sexo con respecto a la seropositividad.

Contacto COVID-19	Serología		Valor P	OR (IC 95%)
	Positivo (%)	Negativo (%)		
Si	12 (13,5)	77 (86,5)	0,45	1,4 (0,6-3)
No	19 (10,1)	169 (89,9)		

Tabla 4. Comparación entre haber tenido contacto con una persona COVID-19 positivo con respecto a la seropositividad.

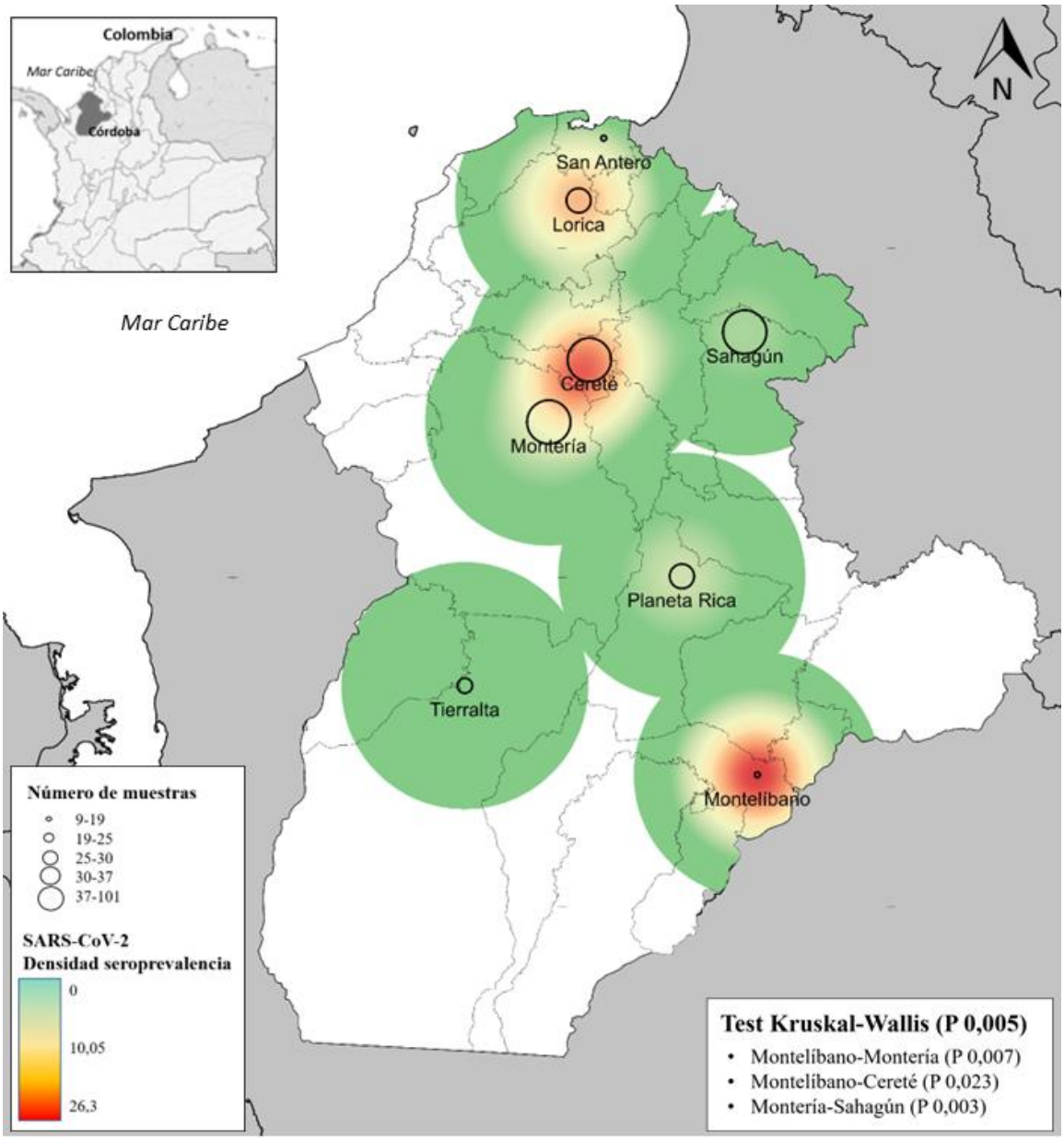


Figura 1. Distribución espacial de la infección por SARS-CoV-2 en perros y gatos del departamento de Córdoba a través de un mapa de calor. Test de Kruskal-Wallis (P 0,005) y comparación por parejas.

Referencias.

1. Bosco-Lauth AM, Hartwig AE, Porter SM, Gordy PW, Nehring M, Byas AD, et al. Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to reexposure in cats. Proc Natl Acad Sci U S A. 2020 Oct 20;117(42):26382–8.

2. Editorial Commentry. The risk of SARS-CoV-2 transmission to pets and other wild and domestic animals strongly mandates a one-health strategy to control the COVID-19 pandemic. One Heal. 2020;10(April):1–4.

3. Hobbs EC, Reid TJ. Animals and SARS-CoV-2: Species susceptibility and viral transmission in experimental and natural conditions, and the potential implications for community transmission. Transbound Emerg Dis. 2021;68(4):1850–67.

4. Bao L, Song Z, Xue J, Gao H, Liu J, Wang J, et al. Susceptibility and Attenuated Transmissibility of SARS-CoV-2 in Domestic Cats. J Infect Dis. 2021;223(8):1313–21.

5. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature. 2020;581(7807):215–20.

6. Sit THC, Brackman CJ, Ip SM, Tam KWS, Law PYT, To EMW, et al. Infection of dogs with SARS-CoV-2. Nature. 2020 Oct 29;586(7831):776–8.

7. Costagliola A, Liguori G, D'angelo D, Costa C, Ciani F, Giordano A. Do animals play a role in the transmission of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)? a commentary. Animals. 2021;11(1):1–11.

8. Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. 2020.

9. Giraldo-Ramirez S, Rendon-Marin S, Jaimes JA, Martinez-Gutierrez M, Ruiz-Saenz J. Sars-cov-2 clinical outcome in domestic and wild cats: A systematic review. Animals. 2021;11(7):1–16.

10. Dileepan M, Di D, Huang Q, Ahmed S, Heinrich D, Ly H, et al. Seroprevalence of SARS-CoV-2 (COVID-19) exposure in pet cats and dogs in Minnesota, USA. Virulence. 2021;12(1):1597–609.

11. Zhang Q, Zhang H, Gao J, Huang K, Yang Y, Hui X, et al. A serological survey of SARS-CoV-2 in cat in Wuhan. Emerg Microbes Infect. 2020 Jan 1;9(1):2013–9.

12. Patterson EI, Elia G, Grassi A, Giordano A, Desario C, Medardo M, et al. Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. Nat Commun. 2020;11(1).

13. Ruiz-Arrondo I, Portillo A, Palomar AM, Santibáñez S, Santibáñez P, Cervera C, et al. 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. Transbound Emerg Dis [Internet]. 2020;00(May):1–4. Available from: wileyonlinelibrary.com/journal/tbed

14. Garay E, Serrano-Coll H, Rivero R, Gastelbondo B, Faccini-Martínez Á, Berrocal J, et al. SARS-CoV-2 in eight municipalities of the Colombian tropics: high immunity, clinical and sociodemographic outcomes. Trans R Soc Trop Med Hyg. 2021;1–9.

CAPÍTULO 5 CONCLUSIONES GENERALES

La infección por SARS-CoV- 2 en perros y gatos de los 8 municipios evaluados del departamento de Córdoba en el periodo de septiembre de 2020 a septiembre de 2021 se caracterizó por una baja seroprevalencia con una tasa de seroconversión alta acorde a los estándares de la prueba comercial utilizada y una baja tasa de infección activa con una prevalencia de las variantes que circulaban en personas COVID-19 positivas en el momento de la toma de muestra. Todas las mascotas positivas tuvieron contacto cercano con al menos una persona con COVID-19. La implementación de métodos de secuenciación de nueva generación o Next-Generation Sequencing (NGS) permitieron identificar un evento de transmisión humano-animal del virus SARS-CoV-2, esto gracias a que el estudio se realizó de forma interdisciplinaria en conjunto con otras investigaciones incluyendo muestras de tutores responsables y personas convivientes con las mascotas.

Se evidenciaron distintos factores que pueden influir en mayor o menor medida la presentación de eventos de transmisión de este virus desde humanos hacia otras especies, como el uso de medidas de aislamiento durante la enfermedad por COVID-19 y la tenencia responsable de las mascotas.

En conclusión, este estudio evidenció la caracterización de la infección por SARS-CoV-2 en animales domésticos viviendo en el departamento de Córdoba y aportó información relevante para motivar nuevas investigaciones implementando la interdisciplinariedad y diferentes métodos serológicos, moleculares y genómicos de vigilancia epidemiológica.

CAPITULO 6 RECOMENDACIONES

- Se recomienda implementar el enfoque One Health y One Welfare en futuras investigaciones.
- Se deben multiplicar los esfuerzos en la vigilancia epidemiológica del virus SARS-CoV-2 en otros hospederos animales no humanos.
- Llevar a cabo estudios acerca de la dinámica de los anticuerpos contra este virus en animales.
- Se deben implementar métodos de secuenciación genómica en la vigilancia de la infección por SARS-CoV-2 en animales en conjunto con los posibles contactos COVID-19 positivos humanos en caso de haber.
- Se deben incrementar los esfuerzos que apunten a la información de las comunidades para evitar o reducir los nuevos casos de SARS-CoV-2 en animales.
- Se recomienda fomentar la tenencia responsable de mascotas, cumpliendo los estándares internacionales de bienestar animal.
- Se recomienda continuar con las medidas de bioseguridad, aislamiento y autocuidado en caso de que el tutor responsable o la mascota presenten un cuadro compatible con COVID-19.
- No abandonar las mascotas en caso de presentar signos compatibles con infección por SARS-CoV-2.

BIBLIOGRAFÍA

1. Alcaldia Mayor de Bogotá D.C.; Secretaria Distrital de Salud; Secretaria Distrital de Ambiente. Política Distrital de Salud para Bogotá D.C. Ambiental 2011-2023 Documento Técnico Línea de Intervención Eventos Transmisibles de Origen Zoonótico [Internet]. Efectos de la contaminacion ambiental. 2011. p. 23. Available from: http://www.facmed.unam.mx/deptos/salud/censenanza/syma/antolog_SaludAmbiental_2013.pdf

2. Vega Aragón RL. Zoonosis emergentes y reemergentes y principios básicos de control de zoonosis. Rev Med Vet (Bogota). 2009;0(17):85–97.

3. Cediel N, de la Hoz F, Villamil LC, Romero J, Díaz A. Epidemiología de la rabia canina en Colombia. Rev Salud Publica. 2010;12(3):368–79.

4. Minsalud, OPS/OMS. Propuesta de Programa Nacional Integral e Integrado de Zoonosis (PNIIZ) SCON2016-00326. 2016;(June):1–170. Available from: https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/VS/PP/SA/Propuesta-intregada-programa-zoonosis.pdf

5. Benavides-Arias D, Soler-Tovar D. Prioritization of zoonotic viral diseases in feral pigs, domestic pigs and humans interface. Biomedica. 2016;36(133):56–68.

6. WHO. Novel Coronavirus (2019-nCoV) SITUATION REPORT - 1. Vol. 1, 21 JANUARY 2020. 2020.

7. Organización Mundial de Sanidad Animal OIE. Ficha Técnica d Enfermedad INFECCIÓN POR SARS-COV-2 EN ANIMALES. Vol. 2. 2021.

8. Cascella M, Rajnik M, Cuomo A, Dulebohn SC, Di Napoli R. Features, Evaluation, and Treatment of Coronavirus [Internet]. StatPearls. 2020. 19 p. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32150360

9. WHO. Coronavirus disease 2019 (COVID-19) Situation Report – 51. Vol. 51, 11 March 2020. 2020.

10. OIE. Sistema Mundial de Información Zoosanitaria [Internet]. Available from: https://wahis.oie.int/#/home

11. Renata CAS, Mariano APM, Maciel BM, Gadelha SR, de Melo Silva M, Belitardo EMMA, et al. First genome sequencing of SARS-CoV-2 recovered from an infected cat and its owner in Latin America. Transbound Emerg Dis. 2021;(January):1–5.

12. Sailleau C, Dumarest M, Vanhomwegen J, Delaplace M, Caro V, Kwasiborski A, et al. First detection and genome sequencing of SARS-CoV-2 in an infected cat in France. Transbound Emerg Dis. 2020;67(6):2324–8.

13. Pagani G, Lai A, Bergna A, Rizzo A, Stranieri A, Giordano A, et al. Human-to-cat sars-cov-2 transmission: Case report and full-genome sequencing

from an infected pet and its owner in Northern Italy. Pathogens. 2021;10(2):1–6.

14. INS Instituto Nacional de Salud. COVID-19 en Colombia [Internet]. Available from: https://www.ins.gov.co/Noticias/paginas/coronavirus.aspx

15. Yen HL, Sit THC, Brackman CJ, Chuk SSY, Gu H, Tam KWS, et al. Transmission of SARS-CoV-2 delta variant (AY.127) from pet hamsters to humans, leading to onward human-to-human transmission: a case study. Lancet. 2022;399(10329):1070–8.

16. Koopmans M. SARS-CoV-2 and the human-animal interface: outbreaks on mink farms. 2020; Available from: https://www.who.int/publications/m/item/who-convened-

17. Ecdc. Detection of new SARS-CoV-2 variants related to mink – 12 November 2020. Stockholm; 2020.

18. Van Dorp L, Tan CCS, Lam SD, Richard D, Owen C, Berchtold D, et al. Recurrent mutations in SARS-CoV-2 genomes isolated from mink point to rapid host-adaptation. bioRxiv. bioRxiv; 2020.

19. Hayashi T, Yaegashi N, Konishi I. Effect of RBD mutation (Y453F) in spike glycoprotein of SARS-CoV-2 on neutralizing antibody affinity. bioRxiv. bioRxiv; 2020.

20. Thanit Sila, Jutapoln Sunghan, Wison Laochareonsuk, Smonrapat Surasombatpattana, Chanon Kongkamol, Thammasin Ingviya, Pisud Siripaitoon, Narongdet Kositpantawong, Siripen Kanchanasuwan, Thanaporn Hortiwakul, Boonsri Charernmak, Ozioma Forstinus Nwabor, Ka and SC. Suspected Cat-to-Human Transmission of SARS-CoV-2, Thailand, July–September 2021. Emerg Infect Dis. 2022;28(7).

21. Costagliola A, Liguori G, D'angelo D, Costa C, Ciani F, Giordano A. Do animals play a role in the transmission of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)? a commentary. Animals. 2021;11(1):1–11.

22. FAO, OIE, OMS. Adopción de un enfoque multisectoria "Una Salud" Guía tripartita para hacer frente a las enfermedades zoonóticas en los países [Internet]. 2019. 180 p. Available from: https://extranet.who.int/sph/docs/file/3857

23. Ghai RR, Wallace RM, Kile JC, Shoemaker TR, Vieira AR, Maria E, et al. A Generalizable One Health Framework for the Control of Zoonotic Diseases. Sci Rep [Internet]. 2022;(September):1–11. Available from: https://doi.org/10.1038/s41598-022-12619-1

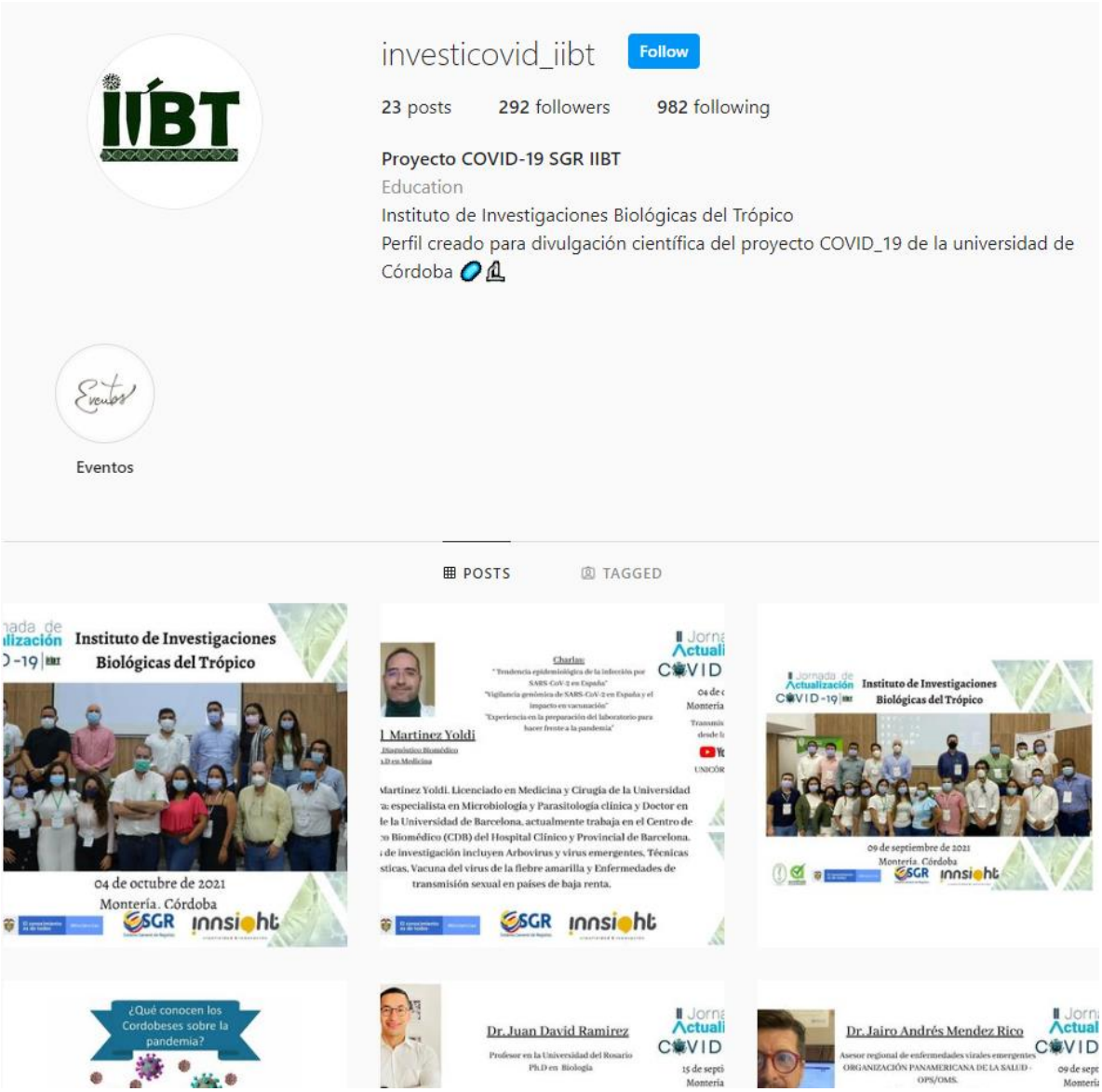
24. (ONU) NU. Objetivos de Desarrollo Sostenible de las Naciones Unidas (ODS) [Internet]. 2015. Available from: https://www.un.org/sustainabledevelopment/%0Asustainable-development-goals

25. Herrero M, Herrera F. ODS en Colombia: Los retos para 2030 [Internet]. 2018. 74 p. Available from: https://www.undp.org/content/dam/colombia/docs/ODS/undp_co_PUBL_julio_ODS_en_Colombia_los_retos_para_2030_ONU.pdf

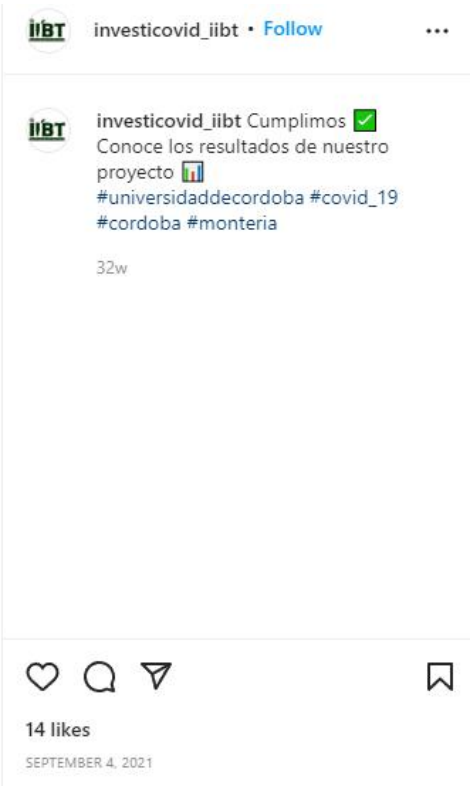
26. Gobernación de Córdoba. Plan de desarrollo 2020-2023: Departamento de Córdoba “Ahora le toca a Córdoba: Oportunidades, Bienestar y Seguridad 2020-2023.” 2020;667.

ANEXOS

Anexo 1. Transferencia de conocimientos a través de redes sociales**,** Instagram investicovid_iibt https://www.instagram.com/investicovid_iibt/?utm_medium=copy_link.



Anexo 2. Transferencia de conocimientos a través de redes sociales**,** Instagram investicovid_iibt https://www.instagram.com/investicovid_iibt/?utm_medium=copy_link.



Anexo 3. Transferencia de información a través de página web, link https://www.controlbox.gatewayti.com.co

Anexo 4. Tabla con características de los animales domésticos evaluados por RT-qPCR

	N=290 (100%)
Especie	
Gato	254 (87,6)
Perro	36 (12,4)
Ciudad	
Sahagún	29 (10)
Montelíbano	13 (4,5)
Lorica	25 (8,6)
Tierralta	23 (7,9)
Planeta Rica	39 (13,4)
Montería	97 (33,4)
San Antero	20 (6,9)
Cereté	42 (15,2)
Positivos RT-qPCR	4 (1,4)

Anexo 5. Características de los animales domésticos evaluados por ELISA

	n=279 (100%)	**n Positivos (%)** 32 (11,5)
Especie		
Gato (*Felis catus)*	195 (69,9)	16 (8,2)
Perro (*Canis familiaris lupus)*	84 (30,1)	16 (19)
Edad		
≤ 1año	135 (48,4)	15 (46,9)
> 1 año	114 (40,9)	14 (43,7)
Desconocido	30 (10,7)	3 (9,4)
Ciudad		
Sahagún	37 (13,3)	2 (5,4)
Montelíbano	19 (6,8)	5 (26,3)
Lorica	30 (10,8)	6 (20,0)
Tierralta	20 (7,2)	0 (0)
Planeta Rica	26 (9,3)	2 (7,7)
Montería	101 (36,2)	9 (8,9)
San Antero	9 (3,2)	0 (0)
Cereté	37 (13,3)	8 (21,6)

Anexo 6. Formato de consentimiento informado utilizado para la toma de muestras de animales

DC 001	Versión 001	FORTALECIMIENTO DE CAPACIDADES INSTALADAS DE CIENCIA Y TECNOLOGÍA DE LA UNIVERSIDAD DE CÓRDOBA PARA ATENDER PROBLEMÁTICAS ASOCIADAS CON AGENTES BIOLÓGICOS DE ALTO RIESGO PARA LA SALUD HUMANA EN EL DEPARTAMENTO DE CÓRDOBA **CONSENTIMIENTO INFORMADO TOMA DE MUESTRAS DE ANIMALES CONVIVIENTES**	
Fecha: 31-08-2020			
Página **1**			

Lugar: _____ Fecha: _____ Cod: _____

Yo ___Nombre y apellidos _____, identificado con: _Tipo y número de identificación _____, con domicilio en ___Municipio _____; tutor responsable de: _Nombre paciente_, especie _____, raza _____. Sexo: F ( ) M ( ), edad _____. He sido invitado para que los animales a mi cargo participen en la investigación.

Manifiesto que he recibido y entendido la información sobre el procedimiento y su técnica ___Nombre del procedimiento_(Anexo 1. 1).

Entiendo que, dentro de los riesgos, posibles consecuencias y efectos colaterales del procedimiento a realizar se encuentran (Anexo 1. 2), y que podrían generarse secuelas como (Anexo 1. 3).

En constancia que _____Nombre paciente_, ha sido valorado/a, que yo he sido interrogado y de haber recibido la información relacionada con procedimiento, de haber aclarado las inquietudes, comprendido la información y de haber leído y comprendido lo consignado en este documento; en mi calidad de propietario/responsable, del paciente procedo a autorizar que mi mascota o animal de compañía participe en esta investigación , y procedo a firmar de manera libre y voluntaria, como constancia de aceptación de los procedimientos. Así mismo sé de mi derecho a rechazar los procedimientos o revocar esta autorización. También entiendo que existen situaciones extraordinarias, cuya probabilidad de ocurrencia es baja, descritas en (Anexo 1. 4) las cuales, he comprendido y aceptado.

Entiendo que no hay beneficios para mi mascota ni para mí, y que no se me recompensará.

Firma _____

Dirección _____

Teléfono _____

Yo ___Nombre y apellidos Veterinario_____ matricula profesional No _____, suscribo este documento como constancia de haber brindado al propietario/ responsable, la información sobre los procedimientos y específicamente los riesgos relacionados con los procedimientos a realizar; quien ha manifestado de manera libre y voluntaria su ___Aceptación o rechazo ___para llevarlos a cabo.

Firma del profesional: _____Matricula: _____

Se crea en _____ el ___ de _____ de 2021.