RESEARCH ARTICLE

REVISED Seroprevalence of exposure to SARS-CoV-2 in domestic dogs and cats and its relationship with COVID-19 cases in the city of Villavicencio, Colombia [version 3; peer review: 2 approved]

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Abstract

Background: Since the beginning of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak, different animal species have been implicated as possible intermediate hosts that could facilitate the transmission of the virus between species. The detection of these hosts has intensified, reporting wild, zoo, farm, and pet animals. The goal of this study was to determine the seroprevalence of anti-SARS-CoV-2 immunoglobulins (IgG) in domestic dogs and cats and its epidemiological association with the frequency of coronavirus disease 2019 (COVID-19) patients in Villavicencio, Colombia.

Methods: 300 dogs and 135 cats were randomly selected in a two-stage distribution by clusters according to COVID-19 cases (positive RT-qPCR for SARS-CoV-2) within the human population distributed within the eight communes of Villavicencio. Indirect enzyme-linked immunosorbent assay (ELISA) technique was applied in order to

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determine anti-SARS-CoV-2 IgG in sera samples. Kernel density estimation was used to compare the prevalence of COVID-19 cases with the seropositivity of dogs and cats.
Results: The overall seroprevalence of anti-SARS-CoV-2 IgG was 4.6% (95% CI=3.2-7.4). In canines, 3.67% (95% CI=2.1-6.4) and felines 6.67% (95% CI=3.6-12.18). Kernel density estimation indicated that seropositive cases were concentrated in the southwest region of the city. There was a positive association between SARS-CoV-2 seropositivity in pet animals and their habitat in Commune 2 (adjusted OR=5.84; 95% CI=1.1-30.88). Spearman's correlation coefficients were weakly positive (p=0.32) between the ratio of COVID-19 cases in November 2020 and the results for domestic dogs and cats from the eight communes of Villavicencio.
Conclusions: In the present research cats were more susceptible to SARS-CoV-2 infection than dogs. This study provides the first positive results of anti-SARS-CoV-2 ELISA serological tests in domestic dogs and cats in Colombia with information about the virus transmission dynamics in Latin America during the COVID-19 pandemic.

Keywords
anthropozoonosis, coronavirus, immunoassay, public health

This article is included in the Emerging Diseases and Outbreaks gateway.

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REVISED **Amendments from Version 2**

The changes for this new version (third) of the manuscript are basic, following the recommendations of the referee regarding better explaining certain aspects of the justification of the study, methodology and improvement in the presentation of results (improvement of Figure 3 and adding the new Table 4).

Any further responses from the reviewers can be found at the end of the article

Introduction

Coronavirus disease 2019 (COVID-19) emerged in the Huanan Seafood Wholesale Market in Wuhan, China, in December 2019.[1] Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was the causal agent of this disease, which was declared as pandemic by the World Health Organisation (WHO) on 11th March 2020.[2] The zoonotic origin of COVID-19 has been evidenced thanks to the high genomic similarity of SARS-CoV-2 with coronaviruses (CoV) recovered from bats[3] and pangolins, the latter considered potential intermediate hosts of the virus.[4] Betacoronaviruses such as SARS-CoV-2 belong to the family Coronaviridae. They exhibit linear single-stranded RNA of positive polarity[5] and cause respiratory and gastrointestinal diseases in mammals.[6] Even though humans are the most frequent route of transmission, it has been reported that cats (*Felis catus*) and dogs (*Canis lupus familiaris*) are susceptible to SARS-CoV-2 infection.[7] This way, reverse zoonosis (anthropozoonosis) is viable through close contact with owners during acute infections.[8]

Since the beginning of the SARS-CoV-2 outbreak, different animal species have been implicated as possible intermediate hosts that could facilitate the virus transmission between species.[9] This is the reason why the determination of these hosts has intensified, evidencing a number of reports involving wild, zoo, farm and pet animals.[10] The zoonotic nature from which the transmission hypothesis has started, determines the importance of investigating animal species considered natural reservoirs of SARS-CoV-2.[11] However, concern for the control and reduction of the spread of the virus has led to more vigorous investigation of the role that pet animals, such as dogs and cats, play in the spread of the disease. Although a cat-to-human transmission case was reported,[12] it has been clearly defined that domestic canines and felines do not play a relevant role in the virus transmission to humans.[8,13]

On the other hand, human-animal transmission has been widely reported.[14,15] This fact generates the need to investigate the implications for public and animal health, taking into account that animals are an epidemiological part of this pandemic.[16] Various epidemiological and experimental studies, through serological detection of antibodies against SARS-CoV-2, neutralising antibodies, and detection of viral genome by reverse transcriptase polymerase chain reaction (RT-qPCR), have confirmed SARS-CoV-2 in pet animals around the world.[17] Likewise, the occurrence of emerging variants has been described, as well as their influence on animals,[18] for example, the Alpha variant (B.1.1.7) in dogs and cats with clinical signs of myocarditis,[19] and the Delta variant (B.1.617.2) in dogs with clinical digestive and respiratory symptoms.[20] Regarding the Omicron variant (B.1.1.529), concluded that the SARS-CoV-2 virus accumulated mutations within host cells in mice, giving rise to the Omicron variant that was transmitted to humans, indicating a '*ping-pong*' (spillover and spillback) evolutionary trajectory between species.[21] Transmission of SARS-CoV-2 Delta variant (AY.127) from hamsters to humans[22] and animal-to-human transmission of SARS-CoV-2 within mink farms[23] have also been reported.

Susceptibility to SARS-CoV-2 is determined by the affinity between the receptor-binding domain (RBD) of the viral spike (S) glycoprotein and the angiotensin-converting enzyme 2 (ACE2) of the host cell. Therefore, since vertebrates have conserved domains of ACE2, transmission of the virus between species becomes possible.[24] Canines have lower susceptibility to SARS-CoV-2 infection in contrast to felines[8,25] that exhibit greater respiratory pathology and efficient transmission of the virus to other felines through aerosols.[26]

In the context of the rapid evolutionary trajectory between species that SARS-CoV-2 has been developing, and taking into account the Report No. 13 of the World Organisation for Animal Health (OIE) of 31st May 2022, which reported 676 outbreaks in animals affecting 23 different species in 35 countries,[27] the need for seroepidemiological monitoring in pet, wild and synanthropic animals becomes essential in order to broadly understand the adaptation, evolution and transmission of SARS-CoV-2.[7]

In Colombia, in December 2021, a lion exhibited symptoms of infection days after being in contact with a COVID-19 positive keeper.[28] However, seroepidemiological studies of exposure to SARS-CoV-2 by pet animals have not been reported to date in the country. Since the dissemination of SARS-CoV-2 in dogs and cats is weak and short-lived, anti-SARS-COV-2 antibody detection studies are the best choice to determine the circulation of this virus in these companion

animals. Of course, the indirect ELISA screening tests that detect immunoglobulin anti-RBD S1 SARS-CoV-2 are called for their greater accuracy in diagnosis.[29] The goal of the present study was to determine the seroprevalence of anti-SARS-CoV-2 immunoglobulins (Ig) class G (IgG) in domestic dogs and cats and its epidemiological association with the frequency of COVID-19 patients in Villavicencio city, Colombia.

Methods

Ethical considerations

This research was endorsed by the Bioethics Committee of the Universidad de los Llanos, according to Minute 02 by consensus of April 6, 2021. In addition, all the owners of the dogs and cats involved in this study signed the respective informed consent.

Type of study and sample size

This is a cross-sectional epidemiological study conducted in Villavicencio, Colombia. It consisted of applying a characterisation survey and taking blood samples from domestic canines and felines. The sample was estimated using the formula for size by proportions in finite populations, using the results obtained by Patterson *et al.*[30] as a reference of p, with SARS-CoV-2 seroprevalences of 3.3% in dogs and 5.8% in cats from Italy. The population assessed in the present study corresponded to 68,651 domestic canines and felines in Villavicencio (47,573 canines and 21,078 felines), according to estimates from the report on anti-rabies vaccination of dogs and cats in Colombia.[31] The confidence interval (CI) considered was 95% and the 'Z' value was 1.96 (1-α). The absolute precision considered was 0.15% (d = 0.0051).

Sampling and inclusion criteria

The participants were selected based on their mandatory participation in the 2021 rabies vaccination campaign, carried out in Villavicencio (capital of Meta department) by the health secretary. A probabilistic sampling was conducted by randomly selected two-stage clusters of domestic dogs and cats from the eight communes (subdivisions) that compose the urban area of Villavicencio (Figure 1), which consisted of the random and proportional selection of individual dogs and cats, the sampling proportion of each cluster was determined according to the frequency of COVID-19 cases (RT-qPCR testing) in each commune[32] (according to Table 1); for this, the EpiInfo v. 3.0 software, from the US Centers for Disease Control and Prevention (CDC) was used (https://www.cdc.gov/epiinfo/esp/es_index.html). The inclusion criteria considered domestic dogs and cats that had lived constantly in their homes for a minimum of two months before starting

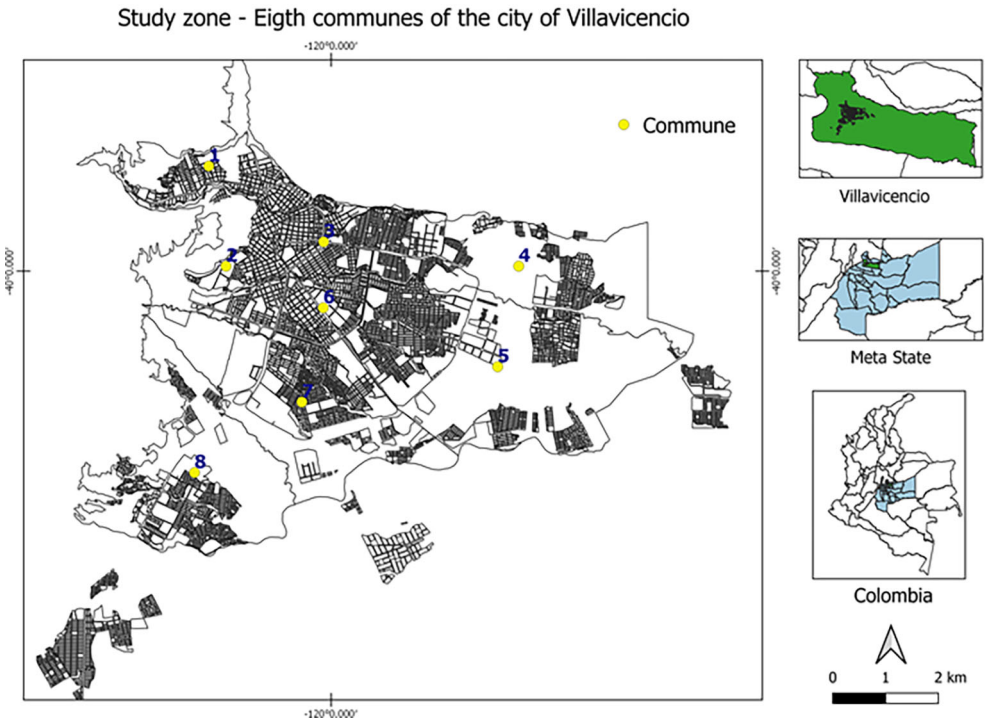

Figure 1. Study zone – eight communes of Villavicencio city, Meta state, Colombia. This figure is an original figure produced by the authors for this article.

Table 1. Pet animal distribution among the eight communes of Villavicencio city according to coronavirus disease 2019 (COVID-19) active cases. Active human cases (RT-qPCR) in November 2020; data from the Villavicencio Municipal Health Secretary (2021).

Commune	COVID-19* active cases (%)	Dogs	Cats	Total pet animals
1	7.4	22	10	32
2	10	30	14	44
3	3.5	10	5	15
4	14.3	43	19	62
5	22.4	68	30	98
6	4.8	14	7	21
7	18.9	58	25	83
8	18.7	55	25	80
Total	100	300	135	435

the present study. We determined as exclusion criteria, the animals that had consumed immunomodulatory medication (e.g., corticosteroid-type immunosuppressants) one week before the sampling were not included in the study.

A total of 435 blood samples were taken (300 domestic canines and 135 domestic felines). For this purpose, the authors of this study collected the blood from the jugular or cephalic vein, previous disinfection of the area with alcohol using a 21-gauge needle or vacutainer. Haemostasis was facilitated by applying pressure with sterile gauze in the sampling site for approximately 30 sec. The samples were centrifuged at 2000 g (Centrifuga Eppendorf 5424R) within three hours after being taken, and the sera were stored at -20 °C until analysis in a freezer (ABBA CVANF502B1). Table 1 shows the representative distribution of 'n' by commune (235 neighbourhoods) in Villavicencio.

Pet animals characterization was performed through a survey applied to the owners, following the model of a SARS-CoV-2 study that involved dogs and cats with COVID-19 patients in a metropolitan area.[33] The characteristics of each pet recorded were: name; sex; age; species; breed; and owners' names. This survey also inquired whether the individuals that cohabited with the pet animals (spontaneous communication) had histories of positive or negative RT-qPCR testing for COVID-19, and one of the survey questions was focused on the possibility for the owner to recognize if whether there were histories of clinical signs of the animals, such as signs in the upper or lower respiratory tract, or non-specific digestive signs (e.g., vomiting, diarrhoea, among others). Coordinates of the houses where the pets lived, were also recorded. The survey can be found as *Extended data*.[57]

Immunoassay

IgG antibodies against the nucleocapsid protein (N) of SARS-CoV-2 in the sera of domestic dogs and cats were qualitatively determined using the indirect enzyme-linked immunosorbent assay (ELISA) (ID Screen® SARS-CoV-2, double antigen multi-species [IDvet, Grabels, France]) according to the manufacturer's instructions. For cats, the kit presents 63% of sensitivity and 96% of specificity. For dogs, the kit has 36% of sensitivity and 85% of specificity. Previous papers used the ID Screen® kit in their studies.[34–37] Each plate contained 96 microwells sensitised with recombinant antigen of purified N protein of SARS-CoV-2, to which the following items were added: two negative controls (NC); two positive controls (PC); and 92 problem sera previously homogenised by vortexing. The optical density (OD) reading was performed using the Cytation 3 multimodal microplate reader (BioTek Instruments, Inc. Winooski, VT, USA) with a wavelength of 450 nm. In total, 435 problem sera samples and 25 pre-pandemic canine sera previously stored at -20 °C were analysed. Using the OD data of each well, the sample/positive control (S/P) ratio was calculated, which was expressed as a percentage using the following formula:

$$S/P\% = \frac{\text{OD sample} - \text{DO NC}}{\text{OD PC} - \text{OD NC}} \times 100$$

The test was validated when the mean OD value of the PC was greater than 0.350, and the ratio of the mean OD values of the PC and NC was greater than three. The samples were considered positive if the S/P ratio was greater than or equal to 60%, doubtful samples or samples in the gray zone had S/P ratios between 50% and 60%, and samples with S/P ratio less than or equal to 50% were considered negative.

Table 2. Characterisation of domestic canines and felines according to their SARS-CoV-2 seropositivity. The following variables were taken into account: species, age, city communes and owners with positive or negative RT-qPCR testing.

		n	%	SARS-CoV-2 seropositivity	Crude OR	95% CI	Adjusted OR	95% CI
Species	Canines	300	69	3.67% (11/300)	1	1	1	1
	Felines	135	31	6.67% (9/135)	1.87	0.76-4.64	2.07	0.78-5.46
Age (Years)	0-5	344	79	4.66% (16/344)	1	1	1	1
	6-10	78	18	3.85% (3/78)	0.85	0.24-2.98	0.92	0.25-3.46
	11-15	13	3	7.69% (1/13)	1.85	0.22-15.2	1.61	0.16-15.93
Communes	1	32	7	0% (0/32)	1	1	1	1
	2	44	10	13.63% (6/44)	4.17*	1.52-11.49	5.84*	1.1-30.88
	3	15	3	6.67% (1/15)	1.48	0.18-11.86	1.97	0.15-25.69
	4	62	14	8.06% (5/62)	2.13	0.75-6.12	3.13	0.57-17.36
	5	98	23	1.02% (1/98)	0.17	0.02-1.29	0.37	0.03-4.2
	6	21	5	4.76% (1/21)	1.02	0.13-8.01	1.96	0.17-23.06
	7	83	19	4.82% (4/83)	1.07	0.35-3.3	2.02	0.36-11.45
	8	80	18	2.5% (2/80)	0.85	0.18-4.67	1.04	0.21-12.21
Owners COVID 19	COVID Test +	25	6	4% (1/25)	0.13	0.01-2.56	0.09	0-2.49
	COVID Test -	410	94	4.63% (19/410)	0.14	0.01-1.43	0.12	0.01-1.56

Note. 95% CI = confidence interval; COVID-19 = coronavirus disease 2019; OR = odds ratio; RT-qPCR = reverse transcriptase polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.
*p <0.05.

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The step-by-step protocol of this trial has been deposited under the title: Immunoassay of SARS-CoV-2 in dogs and cats V.1, DOI: dx.doi.org/10.17504/protocols.io.5qpvorn29v4o/v1 in protocolos.io (https://www.protocols.io/view/immunoassay-of-sars-cov-2-in-dogs-and-cats-v-1-5qpvorn29v4o/v1).

Statistical analysis

The frequencies of the data obtained in the survey and transformation of quantitative variables into categories for their subsequent analysis were estimated. The punctual seroprevalence (P) of SARS-CoV-2 in pet animals in Villavicencio was expressed as a proportion using the following formula, considering 95% CI:

$$P\% = \frac{\#\text{of SARS CoV 2 seropositive cases}}{\text{sample size}} \times 100$$

The risk association measure odds ratio (OR), calculated by the binomial logistic regression model with 95% CI; was used in order to determine whether the frequency of active COVID-19 cases in humans by commune was related to SARS-CoV-2 seropositivity (exposure) of pet animals. Likewise, Spearman correlation was used to establish a possible relationship in the increase of cases in domestic animals at homes with COVID-19. Finally, using Kernel density analysis, the prevalence of COVID-19 in humans by commune was compared to anti-SARS-CoV-2 IgG seropositivity in domestic dogs and cats. A confidence level of 95% was used for all statistical calculations. Statistical estimates were made using the R 4.2 software with the packages dplyr, MASS, corrplot and epiDispaly, and the maps using the QGIS 3.10 software.

Table 3. Animals with a history of respiratory and digestive signs related to SARS-CoV-2 seropositivity.

		No.	%	SARS-CoV-2 seropositivity	95% CI	X^2	*p*-value
Animals with respiratory signs history	Exhibited signs	22	5.1	9.10% (2/22)	2.53-27.81	0.8206	0.365
	Dis not exhibit signs	413	94.9	4.56% (18/395)	2.90-7.09		
Animals with digestive signs history	Exhibited signs	9	2.1	0% (0/9)	0-29.91	NA	NA
	Dis not exhibit signs	426	97.9	4.93% (20/406)	3.21-7.49		

Note. 95% CI = confidence interval; NA = no results are presented because there were no pets detected with digestive signs; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

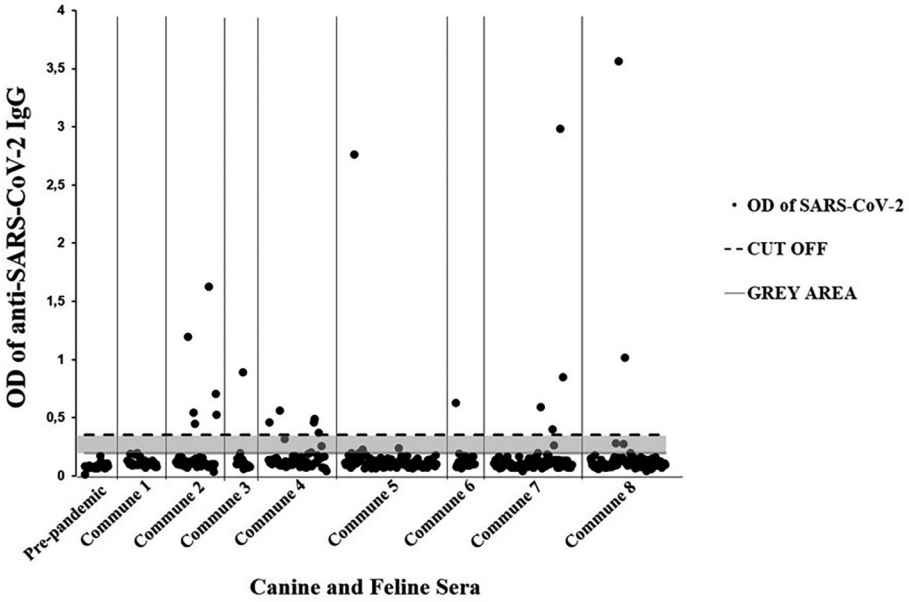

Figure 2. Domestic dogs and cats with SARS-CoV-2 seropositivity from the eight communes of Villavicencio. Ig = immunoglobulin class G; OD = optical density; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Results

The overall seroprevalence of anti-SARS-CoV-2 IgG was 4.60% (95% CI = 3.2-7.4).[57] Specifically, in canines the results indicated 3.67% (95% CI = 2.1-6.4), and in felines 6.67% (95% CI = 3.6-12.18) (Table 2). Twenty seropositive individuals (11 canines and 9 domestic felines) were detected through the enzyme immunoassay. In general, 22 animals with a history of respiratory signs (e.g., cough, runny nose, among others) were detected, of which 9.10% (95% CI = 2.53-27.81) were seropositive for SARS-CoV-2 (Table 3). Additionally, seven immunoassay results were classified as doubtful (gray area). Likewise, all 25 canine pre-pandemic sera were negative (Figure 2).

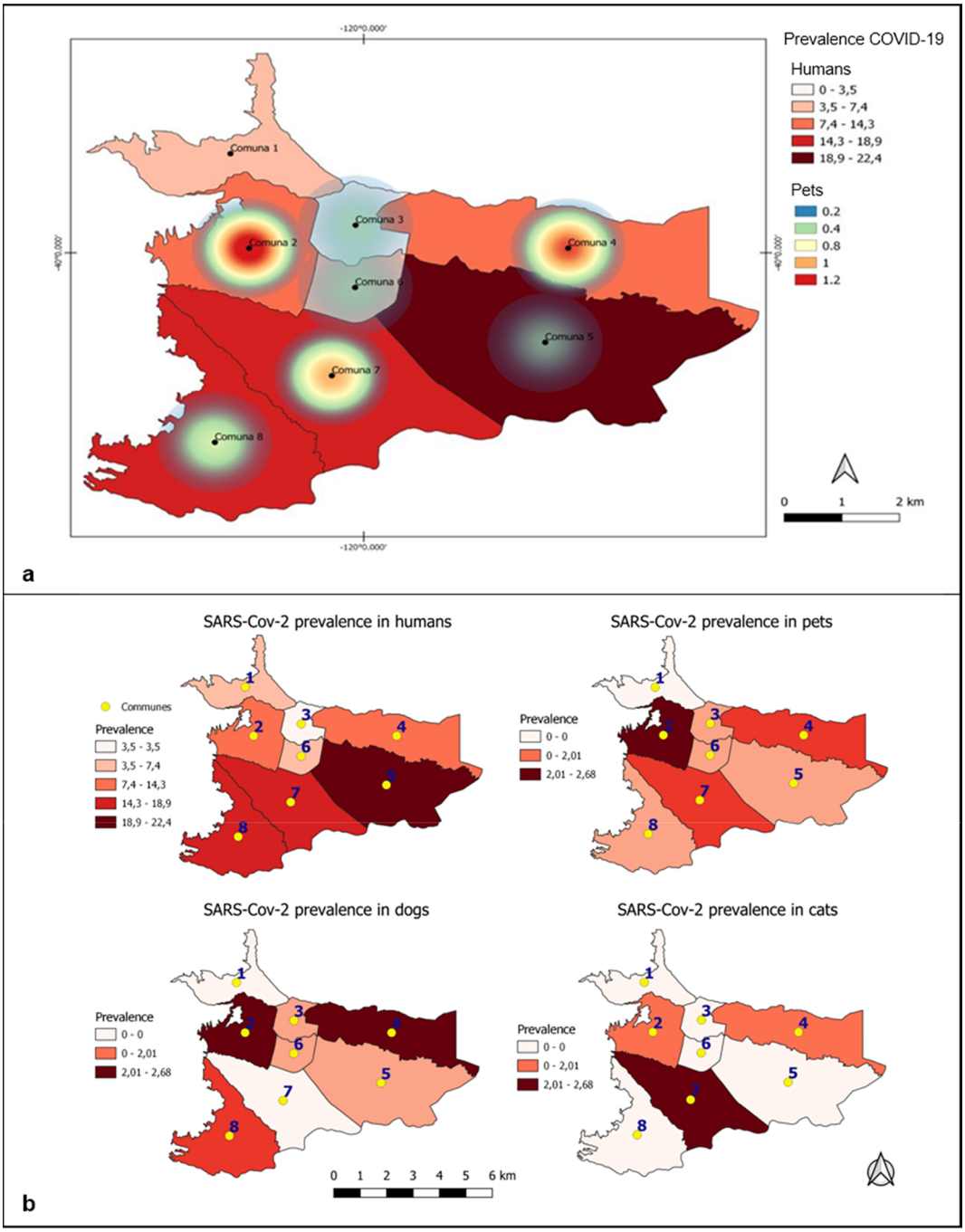

Figure 3. Kernel density and prevalence distribution in humans, dogs and cats in Villavicencio. (a) Kernel density estimate of anti-SARS-CoV-2 IgG prevalence in dogs and cats in Villavicencio. The darkest colored shade represents the highest density of seropositive animals, and the lightest colors represent sites with the lowest densities. (b) Distribution of prevalences in humans, canines, felines and in general of these two domestic species according to each commune. Commune = Commune; COVID-19 = coronavirus disease 2019; IgG = immunoglobulin class G.

Table 4. Spearman correlation between COVID-19 positive cases (RT-qPCR testing) of humans and domestic dogs and cats from the eight Villavicencio communes.

	Humanos	Caninos	Felinos	Mascotas
Caninos	0.01	1	–	–
Felinos	0.33	0.17	1	
Mascotas	0.32	0.68	0.81*	1

**p*-value = 0.01467.

Regarding the eight communes, there was a general seroprevalence of 0, 13.63%, 6.67%, 8.06%, 1.02%, 4.76%, 4.82% and 2.5%, respectively (Table 2). In the map obtained through Kernel density analysis (Figure 3), it is observed that the density of cases was concentrated mainly in the west of the city. Communes with higher densities of SARS-CoV-2 seropositive animals were 2 and 4 in comparison to COVID-19 cases in humans, with a greater number of positive cases in communes 5, 7 and 8. Other visible sites of concentrations of seropositive animals, though with lower density, corresponded to communes 7 and 8. Finally, communes 1, 3, 5 and 6 had densities ranging from low to zero.

Regarding the SARS-CoV-2 exposure and the risk factors analysed, a statistically significant association between SARS-CoV-2 seropositivity and Commune 2 was found (adjusted OR = 5.84; CI 95% = 1.1-30.88). On the other hand, no significant statistical association was found ($p > 0.05$) between anti-SARS-CoV-2 IgG seropositivity and the other items assessed (Table 2). Additionally, among the twenty seropositive animals, only one owner spontaneously confirmed to have positive RT-qPCR result for COVID-19.

Additionally, a Spearman correlation of $p = 0.32$ was found between the ratio of COVID-19 positive cases (RT-qPCR testing) of humans in November 2020 and domestic dogs and cats from the eight Villavicencio communes, result classified as a positive weak correlation. Finally, a strong positive correlation of 0.81 was found between the feline species and their SARS-CoV-2 seropositivity, as well as a positive correlation of 0.68 between the canine species and their SARS-CoV-2 seropositivity (Table 4).

Discussion

In the present study, the seroprevalence of SARS-CoV-2 in canines was 3.67% (11/300) and in felines 6.67% (9/135). Felines had more risk of becoming infected with SARS-CoV-2 that dog (adjusted OR = 2.07; 95% CI = 0.78-5.46) (Table 2) this tendency was no statistically different. In similar studies, Barroso *et al.*[7] found SARS-CoV-2 seroprevalence of 4.7% in dogs and 21.7% in cats in Portugal, determining that, among seropositive animals, 50% had been possibly infected by human-animal transmission. On the other hand, 33.3% of seropositive cats had possibly been infected via the cat-cat route. Colitti *et al.*[38] found a SARS-CoV-2 seroprevalence of 2.3% in dogs and 16.2% in cats in Italy, and Fritz *et al.*[13] found a SARS-CoV-2 seroprevalence of 15.4% in dogs and 23.5% in cats from France. In all the studies mentioned, SARS-CoV-2 prevalence was higher in cats and its transmission was mostly related to exposure to humans when they were more seropositive and more susceptible to infection.[27,38,39]

As a result of the present study, a positive relationship between seropositivity and the age of the animals was observed. The older animals between 11 and 15 years exhibited this tendency predisposition, but it was not statistically different (adjusted OR = 1.61; 95% CI = 0.16-15.93) (Table 2). In this sense, a significant trend was found in the fatality and mortality rates of COVID-19 with advanced age in humans,[40] given that there is a weakened immune system, underlying chronic diseases, multiple drug therapies, lack of attention and self-care, poor environmental hygiene, loneliness, and lack of adequate support from other family members in this population.[41] These reasons could be considered with equal value in the case of animals, especially pet animals.[42] On the other hand, Shi *et al.*[25] reported that three-month-old canines exhibited low susceptibility to experimental infection, contrary to the results obtained in cats, since animals aged less than 100 days and up to nine months were highly susceptible to SARS-CoV-2 infection.

In the present study, no significant differences were found for respiratory and digestive symptoms of the animals sampled according to their SARS-CoV-2 seropositivity ($X^2 = 0.8206$; $p = 0.365$) (Table 3). These results are similar to those reported by Pagani *et al.*[43] and Shi *et al.*[25] i.e., cats infected with SARS-CoV-2 were asymptomatic or highly susceptible to subclinical infections. Contrarily, in Germany, Keller *et al.*[44] reported animals with mainly respiratory symptoms, describing the case of a cat with unresolved pneumonia, which was associated to the owner positive test for COVID-19. SARS-CoV-2-specific nucleic acid analysis was performed, revealing the complete genome and the presence of infection in that patient.

In both canines and felines, the highest seropositivity occurred in Commune 2 (13.63% [6/44]) (Table 2), which is located in the southwest of Villavicencio. Despite the fact that it is a commune with a low population (19,491 inhabitants),[45] it has been reported with the highest number of inhabitants per house (6 inhabitants) in comparison to the other communes,[46] suggesting that having more than one individual infected with SARS-CoV-2 in the same household increased the risk of infection in these pet animals.[38] Likewise, this commune presented a positive association between the seropositivity of the animals sampled (adjusted OR = 5.84; 95% CI = 1.1-30.88) (Table 2) and the seropositivity of the owners, similarly, Colitti *et al.*[38] found a positive association between COVID-19 positive owners and their felines' SARS-CoV-2 seropositivity (OR = 2.5; 95% CI = 1.3-5.2), which may be related to the duration of the pets' exposure to the infected owners, and the close contact of the felines with their owners, suggesting the development of antibodies in domestic animals as a consequence of viral transmission from owners.[38,39,47] In the present study, the association between positive COVID-19 cases (RT-qPCR testing) in humans versus seropositivity in canines and felines from the eight communes of Villavicencio was weakly positive (Spearman's correlation of $p = 0.32$, Table 4). This significance may be influenced by the characterization of the survey, where due to social fear or ignorance some owners could indicate that they were not or had not been positive for the disease, while a strong positive relationship was expected, as in the case of the study conducted by Patterson *et al.*[30] in Italy. On the other hand, the study conducted by Van Aart *et al.*[48] showed that none of the felines had been infected with SARS-CoV-2 despite the fact that these were living with their positive COVID-19 owners. Therefore, these associations between species should be analysed considering different factors.

Animals and humans are susceptible to a large number of different coronaviruses, in fact, it has been shown that all pathogenic human coronaviruses have their origin in animals, which is why studies should focus on their role in the transmission of SARS-CoV-2.[49] In the present study, a human-animal transmission was considered based on the results of Smith *et al.*[50] in the United Kingdom, who ruled out that dogs and cats were reservoirs of infection for humans. However, we cannot be sure about the trasmission direction, which will only be confirmed through further studies. Ultimately, successful elimination of SARS-CoV-2 will only be possible by assessing and controlling transmission in all susceptible animal species, a one health approach that could prevent the re-emergence of the virus in the future.[51,52] Although the 20 canine pre-pandemic sera reacted negatively to the immunodiagnosis, cross-reactions with ancestral coronaviruses in canines and felines are possible, but in low probability when are compared to commercial ELISAs with neutralizing antibody tests.[53,54] The possible cross-reactivity and the need to verify if the reactive antibodies are neutralizing for SARS-CoV-2 are the main limitations of our study; likewise, it is advisable to carry out studies of "SARS-CoV-2 virus neutralization test from the serum bank obtained, since this is the gold-standard test for the Centers for Disease Control and Prevention (CDC).[55]

Conclusion

The present study provides the first positive results of anti-SARS-CoV-2 serological tests (ELISA) in domestic dogs and cats in Colombia, with information about the dynamics of virus transmission in Latin America and the world during the COVID-19 pandemic. As mentioned above, cats were more susceptible to natural SARS-CoV-2 infection than dogs, following similar dynamics described in other studies.[7,38,56] The present study does not provide evidence that domestic canines and felines are sources of infection for humans; however, further studies focused on one health should not be ruled out[52] in order to improve our knowledge about transmission, epidemiology and dynamics of SARS-CoV-2 and promote a better response to possible future pandemics.

Data availability

Underlying data

Figshare: Seroprevalence of exposure to SARS-CoV-2 in domestic dogs and cats and its relationship with COVID-19 cases in the city of Villavicencio, Colombia. https://doi.org/10.6084/m9.figshare.21271137.v2.[57]

This project contains the following underlying data:

- Dataset.xlsx (data on OD IgG Anti-SARS-CoV-2, positive control sample ratio (Indirect ELISA), data on domestic dogs and cats that participated in the project.)

Extended data

Figshare: Seroprevalence of exposure to SARS-CoV-2 in domestic dogs and cats and its relationship with COVID-19 cases in the city of Villavicencio, Colombia. https://doi.org/10.6084/m9.figshare.21271137.v2.[57]

This project contains the following extended data:

- Consent form.docx
- Questionnaire.docx

Acknowledgements

The authors are grateful to the support of the General Research Division of Universidad de los Llanos. Likewise, to professionals and other employees of the Villavicencio Municipal Health Secretary involved in the rabies vaccination program for domestic dogs and cats. An earlier version of this article can be found on SSRN: https://ssrn.com/abstract=4156064 or doi: http://dx.doi.org/10.2139/ssrn.4156064.

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 3

Reviewer Report 13 November 2023

https://doi.org/10.5256/f1000research.152500.r218010

✔ **Laura C. Bohórquez**
FIND, Geneva, Geneva, Switzerland

The authors have presented a prevalence study assessing the exposure to SARS-CoV-2 in domestic animals and its association to the COVID19 cases of their human owners, across 8 territorial entities in the city of Villavicencio, Colombia.

The work is clearly presented and the authors cite relevant current literature.

The study design is appropriate and technically sound; however, I suggest to clarify specifically what is the sample size estimated, and specify if it was estimated per commune. In the Sample size section in Methods the population assessed is referring to the anti-rabies vaccination campaign (68,651) and the formula used and parameters but it does not state the estimated “n” for this study.

In the Statistical analysis section, please specify how the confidence intervals were calculated.

I suggest to the authors to complement in the Methods section the minimization of bias, if it was considered before the study commence. For example, blinding test operators to the positivity status of the owners, selection of the test.

Regarding the discussion please do not repeat all the results text again, and do not refer to the Table X or Figure Y, it is not necessary to duplicate it, that information is already detained in the Results section.

The authors highlighted several times relationships or associations in comparisons that showed to be “not statistically significantly different”. It is not clear why, it seems they focus on the point estimates and do not consider the confidence intervals. I suggest these statements are based on the statistical measures, in that case, stating that there was not a statistical significant association or please state clearly how do you support your conclusions against the statistical evidence.

Keeping in mind what the authors have highlighted, that this study is not assessing the

transmission direction between pets and owners, the authors should be careful in the Discussion, Conclusion and Abstract sections, avoiding the statements such as that the evidence from this study show the dynamics of virus transmission. As it is not the case, I suggest to the authors to rephrase such sentences to, for example, the evidence show the association between sero-positivity in pets and COVID-19 human cases in owners.

I suggest to the authors to complement in the Discussion section the specific limitations of the study (and potential risks of bias), this will enrich their discussion. For example, the selection of the tests, and its performance, because it seems that the performance declared by the manufacturer of the selected test is much higher for cats than for dogs and this could impact the results of this study.

Typos:

- "if whether" in Methods
- "Dis" in Table 3
- "Ig" but it refers to IgG in Figure 2
- Spanish words in Table 4 (caninos, felinos, mascotas)

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Diagnostics, Immunodiagnostics, Molecular Diagnostics, Research and Development of Diagnostics, Clinical Research of Diagnostics and Medical devices.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 15 August 2023

https://doi.org/10.5256/f1000research.152500.r195444

Diego A. Forero
School of Health and Sport Sciences, Fundación Universitaria del Área Andina, Bogotá, Colombia

The authors have incorporated my previous suggestions into the revised manuscript. I have a minor comment: content of table 4 in the current version of the manuscript is in Spanish.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Human health; molecular biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 04 April 2023

https://doi.org/10.5256/f1000research.145608.r167139

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Diego A. Forero
[1] School of Health and Sport Sciences, Fundación Universitaria del Área Andina, Bogotá, Colombia
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The authors took into account several of my previous comments (and it is evident in the revised manuscript). However, several minor aspects highlighted for me in my previous review remain to be considered in the revised manuscript:

- A better description of the rationale of the main aim of the study is needed.
- It is not clear, in the manuscript, whether the authors examined directly some of the animals, for key clinical signs.
- The statistical analysis (statistical tests used to generate a p value) in table 2 is not clear.
- Data for dogs in Figure 3 is still not clear.
- Data for correlation analyses should be presented in the manuscript (or in a supplementary file).
- In the Discussion section, authors must really keep into account the lack of statistical significance of some findings.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Human health; molecular biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have

significant reservations, as outlined above.

Author Response 02 Jul 2023

Dumar Alexander Jaramillo Hernández

1. A better description of the rationale of the main aim of the study is needed.

Change accepted:

R/ In the last paragraph introduction:

Colombia, in December 2021, a lion exhibited symptoms of infection days after being in contact with a COVID-19 positive keeper. 28 However, seroepidemiological studies of exposure to SARS-CoV-2 by pet animals have not been reported to date in the country. *Since the dissemination of SARS-CoV-2 in dogs and cats is weak and short-lived, anti-SARS-COV-2 antibody detection studies are the best choice to determine the circulation of this virus in these companion animals. Of course, the indirect ELISA screening tests that detect immunoglobulin anti-RBD S1 SARS-CoV-2 are called for their greater accuracy in diagnosis*.

Reiners, N, Schnurra, C, Trawinski, H, Kannenberg, J, Hermsdorf, T, Aebischer, A, et al. Performance of a SARS CoV-2 antibody ELISA based on simultaneous measurement of antibodies against the viral nucleoprotein and receptor-binding domain. Eur J Clin Microbiol Infect Dis. (2021) 40:2645–9. doi: 10.1007/S10096-021-04284-5/TABLES/3

2. is not clear, in the manuscript, whether the authors examined directly some of the animals, for key clinical signs.

Change accepted:

R/ In the last paragraph subtitle “Sampling and inclusion criteria”

This survey also inquired whether the individuals that cohabited with the pet animals (spontaneous communication) had histories of positive or negative RT-qPCR testing for COVID-19, and ***one of the survey questions was focused on the possibility for the owner to recognize if*** whether there were histories of clinical signs of the animals, such as signs in the upper or lower respiratory tract, or non-specific digestive signs (e.g., vomiting, diarrhoea, among others)

3. The statistical analysis (statistical tests used to generate a p value) in table 2 is not clear.

R/ To determine the individual significance of each of the predictors introduced in a logistic regression model, the Z statistic and the Wald chi-test are used. Databases are included as a supplement that can be used to corroborate the data. At the epidemiological level, the confidence interval offers more information than the p value.

4. Data for dogs in Figure 3 is still not clear.

Change accepted:

R/ Figure 3 will be change.

5. Data for correlation analyses should be presented in the manuscript (or in a supplementary file).

Change accepted:

R/ The data are included as Table 4 in the article.

6. In the Discussion section, authors must really keep into account the lack of statistical significance of some findings.

R/ Change accepted:

"In the present study, the association between positive COVID-19 cases (RT-qPCR testing) in humans versus seropositivity in canines and felines from the eight communes of Villavicencio was weakly positive (Spearman's correlation of $p = 0.32$). This significance may be influenced by the characterization of the survey, where due to social fear or ignorance some owners could indicate that they were not or had not been positive for the disease, while a strong positive relationship was expected, as in the case of the study conducted by Patterson et al. [29] in Italy. On the other hand, the study by Van Aart et al. [43] showed that none of the felines had been infected with SARS-CoV- despite the fact that these were living with their positive COVID-19 owners. Therefore, these associations between species should be analysed considering different factors."

Only this change is made, since throughout the discussion all the results obtained are taken into account, including the data that are not statistically significant.

Competing Interests: Don't have

Version 1

Reviewer Report 26 January 2023

https://doi.org/10.5256/f1000research.138120.r159713

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Diego A. Forero

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The manuscript describes an analysis of seroprevalence of anti-SARS-CoV-2 IgG in a sample of 300 dogs and 135 cats from a city in Colombia. Several major and minor issues should be addressed:

- A better description of the rationale of the main aim of the study is needed - why are you studying only seroprevalence?
- "SARS-CoV-2 seroprevalence of 9.1% (3.3% in canines and 5.8% in felines)" is not clear (it is not clear that it is additive).
- In "Villavicencio (Meta State)". "state" is not clear, in the context of the organization of Colombia.
- A better description of the recruitment strategy for the current study would be useful.
- In Methods, a better description of "commune" is needed.
- It is not clear whether the authors examined directly some of the animals, for key clinical signs.
- Key parameters, such as specificity and sensitivity, are not described for the ELISA assay used. In addition, in Methods it would be important to include citations to key previous papers using the same kit.
- In Statistical analysis, a description of the specific R packages used is needed.
- Table 2 is incomplete (the header for a group of rows is missing) and the statistical analysis in table 2 is not clear.
- Figure 2 might be presented as supplementary information.
- Figure 3 is not clear - it is not clear what is the signal for humans and what is the signal for animals; in addition authors might use colors in this figure.
- Data for correlation analyses should be presented.
- In the Discussion section, authors must keep into account the lack of statistical significance of some findings.
- Possible cross-reactivity of the ELISA assay is not discussed in the manuscript.
- Limitations of the current study and recommendations for future studies are needed.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Human health; molecular biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 10 Feb 2023

Dumar Alexander Jaramillo Hernández

1. A better description of the rationale of the main aim of the study is needed - why are you studying only seroprevalence?

R/ One of the main limitations of our study is the lack of economic support. We could have done more advanced studies such as seroincidence, detection of neutralizing antibodies, or isolation of viral genomic material. Nevertheless, we just study seroprevalence in order to include an important and representative sample size of cats and dogs in Villavicencio.

2. "SARS-CoV-2 seroprevalence of 9.1% (3.3% in canines and 5.8% in felines)" is not clear (it is not clear that it is additive).

R/ Change accepted:
Methods
Type of study and sample size paragraph
Line 2 “The sample was estimated using the formula for size by proportions in finite populations, using the results obtained by Patterson *et al*.[29] as a reference of p, with SARS-CoV-2 seroprevalences of 3.3% in dogs and 5.8% in cats from Italy”.

3. In "Villavicencio (Meta State)". "state" is not clear, in the context of the organization of Colombia.

R/ Colombia is divided into 32 departments and one capital district (Bogotá). Each Department has a capital city. In the case of the Meta Department, the capital city is Villavicencio.
Change accepted: The word "State" will be changed for Department on each occasion.

E.g. **Methods**
Sampling and inclusion criteria paragraph
Line 1 "The participants were selected based on their mandatory participation in the 2021 rabies vaccination campaign, carried out in Villavicencio (capital of Meta department) by the health secretary".
However, in some publications the word "state" means "departamento".

4. A better description of the recruitment strategy for the current study would be useful.

R/ The inclusion and exclusion criteria used in order to estimate the sample size are mentioned under the subheading "Sampling and inclusion criteria".

Change accepted:
The subheading will change to "Sampling, inclusion, and exclusion criteria"
The sentence "The animals that had consumed immunomodulatory medication (e.g., corticosteroid-type immunosuppressants) one week before the sampling were not included in the study." Will be changed for:
"We determined as exclusion criterion, the animals that had consumed immunomodulatory medication (e.g., corticosteroid-type immunosuppressants) one week before the sampling."

5. In Methods, a better description of "commune" is needed.

R/ In Colombia, Departments are subdivided into municipalities which are in turn subdivided into "corregimientos" in rural areas and into "comunas" (communes) in urban areas. Each commune, in turn is divided into neighborhoods.

Change accepted:
Methods
Sampling and inclusion criteria paragraph
Line 2 "A probabilistic sampling was conducted by randomly selected two-stage clusters of domestic dogs and cats from the eight communes (subdivisions) that compose the urban area of Villavicencio (Figure 1), …".

6. It is not clear whether the authors examined directly some of the animals, for key clinical signs.

R/ No, animals were randomly selected among those who participated in the rabies vaccination campaign. Once these were selected, a survey was applied, and one of the questions was related to the clinical signs.

7. Key parameters, such as specificity and sensitivity, are not described for the ELISA assay

used. In addition, in Methods it would be important to include citations to key previous papers using the same kit.

R/ Change accepted:
Methods
Immunoassay paragraph
Line 1. IgG antibodies against the nucleocapsid protein (N) of SARS-CoV-2 in the sera of domestic dogs and cats were qualitatively determined using the indirect enzyme-linked immunosorbent assay (ELISA) (ID Screen® SARS-CoV-2, double antigen multi-species [IDvet, Grabels, France]) according to the manufacturer's instructions. For cats, the kit presents 63% of sensitivity and 96% of specificity. For dogs, the kit has 36% of sensitivity and 85% of specificity. Previous papers used the ID Screen® kit in their studies (48-51).

References
48. Bessière P, Vergne T, Battini M, Brun J, Averso J, *et al*.: **SARS-CoV-2 Infection in Companion Animals: Prospective Serological Survey and Risk Factor Analysis in France**. *Viruses*. 2022; **14**(1178). PubMed Abstract I Publisher Full Text

49. Diezma-Díaz C, Álvarez-García G, Regidor-Cerrillo J, Miró G, Villanueva-Saz S, *et al*.: **A comparative study of eight serological methods shows that spike protein-based ELISAs are the most accurate tests for serodiagnosing SARS-CoV-2 infections in cats and dogs**. *Front. Vet.* 2023. Sci. **10**(1121935). Publisher Full Text

50. Barua S, Hoque M, Adekanmbi F, Kelly P, Jenkins-Moore M, *et al*.: **Antibodies to SARS-CoV-2 in dogs and cats, USA**. *Emerg Microbes Infect.* 2021. **10**(1):1669-1674. PubMed Abstract I Publisher Full Text

51. Neira V, Brito B, Agüero B, Berrios F, Valdés V, *et al*.: **A household case evidences shorter shedding of SARS-CoV-2 in naturally infected cats compared to their human owners**. *Emerg Microbes Infect*. 2021. **10**(1):376-383. PubMed Abstract I Publisher Full Text

8. In Statistical analysis, a description of the specific R packages used is needed.

R/ Change accepted:
Methods
Statistical analysis paragraph
Line 9. Statistical estimates were made using the R 4.2 software with the packages dplyr, MASS, corrplot and epiDispaly, and the maps using the QGIS 3.10 software.

9. Table 2 is incomplete (the header for a group of rows is missing) and the statistical analysis in table 2 is not clear.

R/ In theTable 2, the header of the row 1, 2, 3, 4 ... 8: is “Communes”. This header is included in the original manuscript.

On the other hand, statistical analysis included in table 2 are described in the fourth paragraph of results:

Line 1 “Regarding the SARS-CoV-2 exposure and the risk factors analysed, a statistically significant association between SARS-CoV-2 seropositivity and Commune 2 was found (adjusted OR = 5.84; CI 95% = 1.1-30.88).”

10. Figure 2 might be presented as supplementary information.

R/ Change not accepted, it is important to show the OD distribution among communes, showing the positive results and grey area results.

11. Figure 3 is not clear - it is not clear what is the signal for humans and what is the signal for animals; in addition authors might use colors in this figure.

R/ Change accepted:
Results
Figure 3 will be change by the colored one.

12. Data for correlation analyses should be presented.
R/ Change not accepted, here we present a table with the data for the correlation analyses about the association between positive COVID-19 cases (RT-qPCR testing) in humans versus seropositivity in pets. Nevertheless, it is not necessary to show the table in the paper, since we mention the important data in the discussion (paragraph 4, line 10).

	Human	Dogs	Cats	Pets
Dogs	0,01	1	--	--
Cats	0,33	0,17	1	
Pets	0,32	0,68	0,81*	1

**p*-value = 0.01467

13. In the Discussion section, authors must keep into account the lack of statistical significance of some findings.

R/ In the results and discussion sections, we mentioned and pointed out that results were not significant (just the results associated with commune 2 were statistically significant); however, there were trends that correlate with other studies and we mentioned them explaining that these were not significant (Eg. Discussion paragraph 2, line 1):
“As a result of the present study, a positive relationship between seropositivity and the age of the animals was observed. The older animals between 11 and 15 years exhibited this tendency predisposition, but it was not statistically different”.

14. Possible cross-reactivity of the ELISA assay is not discussed in the manuscript.

R/ Change accepted: The following paragraph will be added to the discussion section.
Discussion
Although the 20 canine pre-pandemic sera reacted negatively to the immunodiagnosis, cross-reactions with ancestral coronaviruses in canines and felines are possible, but in low probability when are compared to commercial ELISAs with neutralizing antibody tests (54,

55).

54. Perera R, Ko R, Tsang O, Hui D, Kwan M *et al.*: **Evaluation of a SARS-CoV-2 surrogate virus neutralization test for detection of antibody in human, canine, cat, and hamster sera.** *J Clin Microbiol*. 2021;59(2):e02504–20. PubMed Abstract I Publisher Full Text

55. Hughes EC, Amat JAR, Haney J, Parr J, Logan N, *et al.*: **SARS-CoV-2 serosurveillance in a patient population reveals differences in virus exposure and antibody-mediated immunity according to host demography and healthcare setting**. *J Infect Dis*. 2021;223(6):971-980. PubMed Abstract I Publisher Full Text

15. Limitations of the current study and recommendations for future studies are needed.

R/ Change accepted: The following paragraph will be added to the discussion section.
Discussion
The possible cross-reactivity and the need to verify if the reactive antibodies are neutralizing for SARS-CoV-2 are the main limitations of our study; likewise, it is advisable to carry out studies of SARS-CoV-2 neutralization from the serum bank obtained, since this is the gold-standard test for the Centers for Disease Control and Prevention (CDC) (56).

56. Centers for Disease Control and Prevention. **Coronavirus disease 2019 (COVID-19) 2020 interim case definition, approved April 5, 2020.** 2023 [cited 2023 February 9]. Reference Source

Competing Interests: The authors declare no conflict of interest.