

1 **The Omicron (B.1.1.529) SARS-CoV-2 variant of concern also affects**  
2 **companion animals**

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8 **Abstract**

9 The recent emergence of the Omicron variant (B.1.1.529) has brought with it a large increase  
10 in the incidence of SARS-CoV-2 disease worldwide. However, there is hardly any data on the  
11 incidence of this new variant in companion animals. In this study, we have detected the  
12 presence of this new variant in domestic animals such as dogs and cats living with owners  
13 with COVID19 in Spain that have been sampled at the most optimal time for the detection of  
14 the disease. None of the RT-qPCR positive animals (10.13%) presented any clinical signs and  
15 the viral loads detected were very low. In addition, the shedding of viral RNA lasted a short  
16 period of time in the positive animals. Infection with the Omicron variant of concern (VOC)  
17 was confirmed by a specific RT-qPCR for the detection of this variant and by sequencing.  
18 These outcomes suggest a lower virulence of this variant in infected cats and dogs. This study  
19 demonstrates the transmission of this new variant from infected humans to domestic animals  
20 and highlights the importance of doing active surveillance as well as genomic research to  
21 detect the presence of VOCs or mutations associated with animal hosts.

## 22 **Introduction**

23 The pandemic associated with the COroNaVirus Disease 2019 (COVID-19), produced by the  
24 SARS-CoV-2 virus, has been active for almost two years now. From the beginning, 406  
25 million cases have been confirmed in the world, with 6.09 million deaths according to the last  
26 World Health Organization (WHO) report (World Health Organisation, 2022).

27 The SARS-CoV-2 is an RNA virus whose organization is shared with other *Beta*  
28 coronaviruses. The genome of this virus consists of 13 opening reading frames (ORFs) and  
29 15 non-structural proteins (NSP). The ORFs, from 5' to 3', codify for replicase  
30 (ORF1a/ORF1b), spike (S) protein, envelope (E) protein, membrane (M) protein, and  
31 nucleocapsid (N) protein (Hu, Guo et al. 2021, Raj 2020; Viralzone, 2022). Due to these  
32 genomic characteristics, this coronavirus has been suffering a great number of mutations,  
33 mainly in the spike protein, which may have influenced the virus transmission rate, the  
34 disease severity, or abrogate the immunity produced by the vaccines, among other factors  
35 (Zou, Xia et al. 2021). The apparition of these mutations has triggered virus evolution.  
36 Consequently, new variants have been identified. According to the pathogenic potential and  
37 the virulence of the different isolates, the WHO have classified them into variants of concern  
38 (VOCs), variants of interest (VOIs), and variants under monitoring (VUMs) (He, Hong et al.  
39 2021). Until December 2021, four VOCs had been reported: Alpha (B.1.1.7), Beta (B.1.351),  
40 Gamma (P.1), and Delta (B.1.617.2) (Saxena, Kumar et al. 2021). On November 26<sup>th</sup>, a new  
41 variant was determined by the WHO as the 5<sup>th</sup> VOC, named Omicron (B.1.1.529). The first  
42 sample identified as this variant was taken in South Africa on the 9<sup>th</sup> of November, while the  
43 first sequenced case was from a sample collected in Botswana on the 11<sup>th</sup> of November (He,  
44 Hong et al. 2021).

45 Until now, the Omicron variant is the VOC with the largest number of mutations detected,  
46 with 34 of them accumulated in the spike protein. Several of these mutations in the spike

47 protein have been related to increased viral antibody neutralization evasion capacity or higher  
48 affinity between the spike/ACE receptor binding (Zou, Xia et al. 2021), facilitating the virus  
49 entry into the cell. Thus, this constellation of mutations appears to have influenced virus  
50 transmissibility, severity, and immune evasion (CDC 2021, He, Hong et al. 2021, Araf, Akter  
51 et al. 2022). These mutations have led to greater contagiousness than the previous variants as  
52 well as different clinical signs, which consist of slight fever, myalgia, fatigue, and shortness  
53 of breath. However, the most dangerous characteristic of this variant is its high rate of  
54 immune escape even in previously immunized by natural infection and vaccinated people  
55 (Meo, Meo et al. 2021). Because of these characteristics, the Omicron variant has gained  
56 great concern in public health worldwide.

57 In Spain, according to data published by the Ministry of Health, the cumulative incidence of  
58 SARS-CoV-2 rose from 77 cases per 100,000 inhabitants on 15<sup>th</sup> November 2021 to 465 on  
59 15<sup>th</sup> December 2021, showing a significant increase. It continued growing until reaching  
60 3,418 on January 20<sup>th</sup>, 2022. This growth in cases coincided with the dates on which the new  
61 Omicron variant was first detected in Spain, around mid-December 2021. As could be  
62 expected, sequence analysis since that period has revealed the growing dominance of the new  
63 VOC in the country (Ministerio de Sanidad de España, 2021).

64 Within the Omicron variant, three lineages or subvariants are distinguished so far:  
65 B.1.1.529.1 (BA.1), B.1.1.529.2 (BA.2), and B.1.1.529.2 (BA.3). BA.2 lineage has 32  
66 mutations shared with BA.1, but 28 distinct, and BA.3 spike protein is a combination of BA.1  
67 and BA.2 with no new mutations. The most prevalent is the BA.1 sublineage, while BA.2 has  
68 been observed to reinfect patients previously infected with BA.1, being more prevalent in  
69 Denmark (Chen and Wei 2022; Mohapatra, Kandi et al. 2022; Desingu and Nagarajan, 2022).

70 Shortly after the SARS-CoV-2 virus entered our lives, field studies on the incidence in  
71 animals, as well as experimental studies, began to be carried out to learn about their role in  
72 this new disease (Shi, Wen et al. 2020). In the case of animals, several studies have found  
73 that some species such as *Canis lupus familiaris*, *Neovison vison*, *Manis javanica*,  
74 *Mesocricetus auratus* and *Odocoileus virginianus* are susceptible to the Omicron variant, as  
75 revealed by sequencing results from natural or experimental infection (GISAID). However,  
76 experimental studies on hamsters have evidenced the lower pathogenicity of this variant in  
77 comparison with Delta and B.1.1 variants in this species, based on different variables such as  
78 body weight and respiratory function (Suzuki, Yamasoba et al. 2022). The conclusions  
79 obtained by these studies were that, unlike other VOCs, Omicron is not able to efficiently  
80 replicate in the lower respiratory tract of Syrian hamsters, which results in the detection of  
81 lower viral loads and fewer pathology findings in the lungs of the experimentally infected  
82 animals comparing with infection by other isolates (Abdelnabi, Foo et al. 2022). In addition,  
83 inoculated mice with B.1.1.529 (Omicron) had lower levels of pro-inflammatory cytokines  
84 and chemokines, on occasions similar to non-infected mice, than those inoculated with the  
85 B.1.351 (Beta) variant (Halfmann et al. 2022).

86 Despite these results suggesting a lower virulence of this variant in infected animals, very  
87 different results were observed in an experimental study in wild carnivores (mink, *Neovison*  
88 *vison*) which are known to be very susceptible to SARS-CoV-2 virus infection. In this study,  
89 minks were infected with the Omicron variant. The animals became ill, had clinical  
90 symptoms, positive PCR results, as well as macroscopic and microscopic lesions post mortem  
91 (Virtanen, Aaltonen et al. 2022). All these aspects lead us to wonder what will be the  
92 relevance of the infection with the Omicron variant in species in close contact with humans  
93 such as dogs and cats in comparison with the previously described VOCs (Barroso-Arevalo,  
94 Rivera et al. 2021, Barroso-Arévalo, Sánchez-Morales et al. 2022, Doerksen, Lu et al. 2021).

95 Is transmissibility to susceptible pets higher with this variant, as is occurring in the case of  
96 humans? What are the clinical repercussions of the infection in cats and dogs? To elucidate  
97 the implications of infection with the Omicron variant in pets, we have carried out an active  
98 sampling of cats and dogs in close contact with SARS-CoV-2 infected people with clinical  
99 signs compatible with this variant and/or confirmed by RT-qPCR or sequencing. In this  
100 study, we have observed a low prevalence of infection in the animals, as well as low viral  
101 loads in the positive cases, despite the samplings being carried out at the optimum moment to  
102 detect human-to-pet transmission.

## 103 **Material and methods**

### 104 *Animal and owner sample collection*

105 Samples from domestic animals including cats (n=28), dogs (n=50), and rabbit (n=1) were  
106 taken between the 15<sup>th</sup> of December 2021 to 24<sup>th</sup> of March 2022. A total of 69 animals (21  
107 cats and 47 dogs) were from Madrid, 6 animals (3 cats and 3 dogs) from Galicia, and 4 cats  
108 (from the Basque Country). All these animals were sampled during the quarantine period of  
109 their owner and, therefore, had been in contact with positive people for SARS-CoV-2. The  
110 samples were taken using protocols approved by the Complutense University of Madrid's  
111 Ethics Committee for Animal Experiments (Project License 14/2020). Owners were informed  
112 about the purpose of the study as well as the data protection policy. When possible, samples  
113 were taken on consecutive days to gather more information about the potential animal  
114 infection. The samples consisted of oral/nasal and rectal swabs collected in DeltaSwab® Virus  
115 containing 3ml of viral transport media (MTV) (Deltalab S.L., Cataluña, Spain) and sera if  
116 possible that were collected in tubes without anticoagulant. All the samples were refrigerated  
117 and taken to the Health Surveillance Centre (VISAVET) at the Complutense University of  
118 Madrid and stored at -80°C until analysis. In addition, a survey of the owners was carried out

119 in order to know the potential symptoms they were presenting to confirm Omicron variant  
120 associated signs, as well as a nasal swab sample collection in some cases to confirm the  
121 SARS-CoV-2 variant involved in the infection by RT-qPCR and sequencing.

122 *Detection of SARS-CoV-2 infection by reverse transcription-quantitative PCR (RT-qPCR)*  
123 *and specific Omicron RT qPCR and virus isolation*

124 Total RNA was extracted using the column-based High Pure Viral Nucleic Acid Kit (Roche,  
125 Basel, Switzerland), according to the manufacturer's instructions. Total RNA was suspended  
126 in RNase/DNase-free water and stored at -80°C. The detection of the RNA of SARS-CoV-2  
127 was carried out using a diagnostic RT-qPCR, hereafter "Diagnosis PCR", based on the  
128 detection of the envelope protein (E)-encoding gene (Sarbeco) and two targets (IP2 and IP4)  
129 of the RNA-dependent RNA polymerase gene (RdRp) in an RT-qPCR protocol established  
130 by the World Health Organization according to the guidelines that can be found at  
131 [https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance)  
132 [guidance/laboratory-guidance](https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance) (Corman, Landt et al. 2020).

133 An specific RT-qPCR was used for the identification of the SARS-CoV-2 Omicron variant,  
134 hereafter "Omicron PCR", targeting both the envelope protein (E) - encoding gene as well as  
135 an Omicron-specific spike insertion-deletion mutation (indel\_211-214) found in the  
136 B.1.1.529/BA.1 lineage and BA.1.1 sublineage, so in the case of the BA.2 and BA.3 Omicron  
137 lineages would only be detected by the gen E target. The kit used was the SuperScript III  
138 Platinum One-Step qRT-PCR kit (Invitrogen) according to the protocol described in (Sibai,  
139 Wang et al. 2022).

140 Positive samples for RT-qPCR were subjected to attempts of viral isolation using the  
141 previously described methods in (Gortázar, Barroso-Arévalo et al. 2021).

142 *Whole-genome sequencing and phylogenetic analysis*

143 Whole-genome sequences were obtained from the two positive oropharyngeal swabs samples  
144 with the higher viral loads based on Ct values (Ct of 32.45 and 30.01) by both “Diagnosis”  
145 and “Omicron” RT-qPCRs, following the protocol described by (Paden, 2020). Sequence  
146 analysis was performed using the Sequencing Analysis software v.5.3.1(Applied  
147 Biosystems), while SeqScape v.2.5 software (Applied Biosystems) was used for sequence  
148 assembly using the SARS-CoV-2 isolate Wuhan-Hu-1, complete genome (GenBank  
149 accession number: NC\_045512) as a reference genome.

150 Phylogenetic analysis was performed using MEGA X software (Tamura, 1992). Four  
151 sequences were obtained from this study (Dog\_8, Cat\_19, Owner\_1, and Owner\_2, which  
152 correspond with one dog, one cat, the dog’s owner, and the owner of Cat\_26, 27, and 28.  
153 Unfortunately, no positive sample for sequencing was available from the owner of Cat\_19. In  
154 the case of cats 26, 27, and 28, sequencing was not possible because of the low RNA loads of  
155 the positive samples (Table 1).

156 A total of 31 additional representative sequences were used for the analysis, including  
157 sequences from cats and dogs, the reference genome from Wuhan, as well as variants of  
158 concern such as the B.1.1.7 variant from the United Kingdom, variant B.1.35 from South  
159 Africa, variant B.1.617.2 from India, variant B.1.1.248 from Brazil and lineages BA.1 and  
160 BA.2 of the B.1.1.529 Omicron variant.

161 The final alignment involved 35 whole-genome sequences with an average amino acid p-  
162 distance (1-amino acid identity) lower than 0.001, which is considered adequate since it is  
163 within the acceptance threshold of <0.8 (Tamura, 1992). This alignment was used to build the  
164 phylogenetic tree using the maximum likelihood method and bootstrap testing of 2,000  
165 replicates. The best model was the Tamura-Nei Model, so it was the one used to create the  
166 phylogenetic tree.

167 *Virus neutralization test (VNT) for detection of specific neutralizing antibodies against SARS-*  
168 *CoV-2*

169 Virus neutralization test (VNT) was used to confirm the presence of neutralizing antibodies  
170 against SARS-CoV-2 in all the sera collected.

171 Briefly, the VNT was performed in duplicate in 96-well plates by incubating 25  $\mu$ L of two-  
172 fold serially diluted sera with 25  $\mu$ L of 100 TCID<sub>50</sub>/ml of SARS-CoV-2. The virus-serum  
173 mixture was incubated at 37°C with 5% CO<sub>2</sub>. At 1-hour post-incubation, 200  $\mu$ L of Vero E6  
174 cell suspension were added to the virus-serum mixtures, and the plates were incubated at  
175 37°C with 5% CO<sub>2</sub>. The neutralization titers were determined at 3 days post-infection. The  
176 titer of a sample was recorded as the reciprocal of the highest serum dilution that provided at  
177 least 100% neutralization of the reference virus, as determined by the visualization of  
178 cytopathic effect (CPE). In addition, at the end of the period (3 days post-infection), cells  
179 were fixed with 6% paraformaldehyde and then stained with crystal violet to observe the  
180 cytopathic effect.

## 181 **Results**

### 182 *Sampling data*

183 A total of 50 dogs, 28 cats, and 1 rabbit (n=79) were sampled during the quarantine period of  
184 their owners from 15th December 2021 to 23<sup>h</sup> March 2022, coinciding with the period of the  
185 highest prevalence of the Omicron variant in Spain (Ministerio de Sanidad de España., 2022)  
186 in Madrid (n=69), Galicia (n=6) and the Basque Country (n=4).

187 All the owners reported a high level of contact with the pets included in the sampling during  
188 their confinement period. None of the animals showed any compatible symptoms with the  
189 illness.



190 *SARS-CoV-2 infection prevalence*

191 SARS-CoV-2 RNA was detected by RT-qPCR in seven cats and one dog by both “Diagnosis  
 192 PCR” and “Omicron PCR”. This represents 10.13% of the total analyzed animals. All of the  
 193 positive animals were sampled in Madrid. All the positive samples for RT-qPCR were  
 194 negative for viral isolation (Table 1).

195 Table 1. Positive animals to RT-qPCR including specie, date of sampling, sample type, the  
 196 day post-infection of the owner (DPI), RT-qPCR Cycle threshold (Ct) value in both  
 197 “Diagnosis PCR” and “Omicron PCR”, sequence if available, and viral isolation result.

Animal, date of collection	Sample type	DPI owner	Diagnosis RT-qPCR Ct	Omicron RT-qPCR Ct	Sequence	Viral isolation
Cat_2, 20th December, 2021	Rectal swab	2 DPI	34.2	36.85	NA	Negative
Dog_8, 1 <sup>st</sup> January, 2022	Oropharyngeal swab	3 DPI	32.45	36.92	B.1.1.529	Negative
Cat_7, 16 <sup>th</sup> January, 2022	Oropharyngeal swab	2 DPI	36.6	38.03	NA	Negative
Cat_19, 22 <sup>th</sup> January, 2022	Oropharyngeal swab	3 DPI	30.01	32.78	B.1.1.529	Negative
Cat_13, 27 <sup>th</sup> January, 2022	Oropharyngeal swab	4 DPI	35.7	37.67	NA	Negative
Cat_26, 18 <sup>th</sup> March, 2022	Oropharyngeal swab	2 DPI	34.5	39.65	NA	Negative
Cat_27, 18 <sup>th</sup> March, 2022	Oropharyngeal swab	3 DPI	35.6	39.05	NA	Negative
Cat_28, 19 <sup>th</sup> March, 2022	Oropharyngeal swab	4 DPI	35	37.86	NA	Negative

198 \*NA: Not available

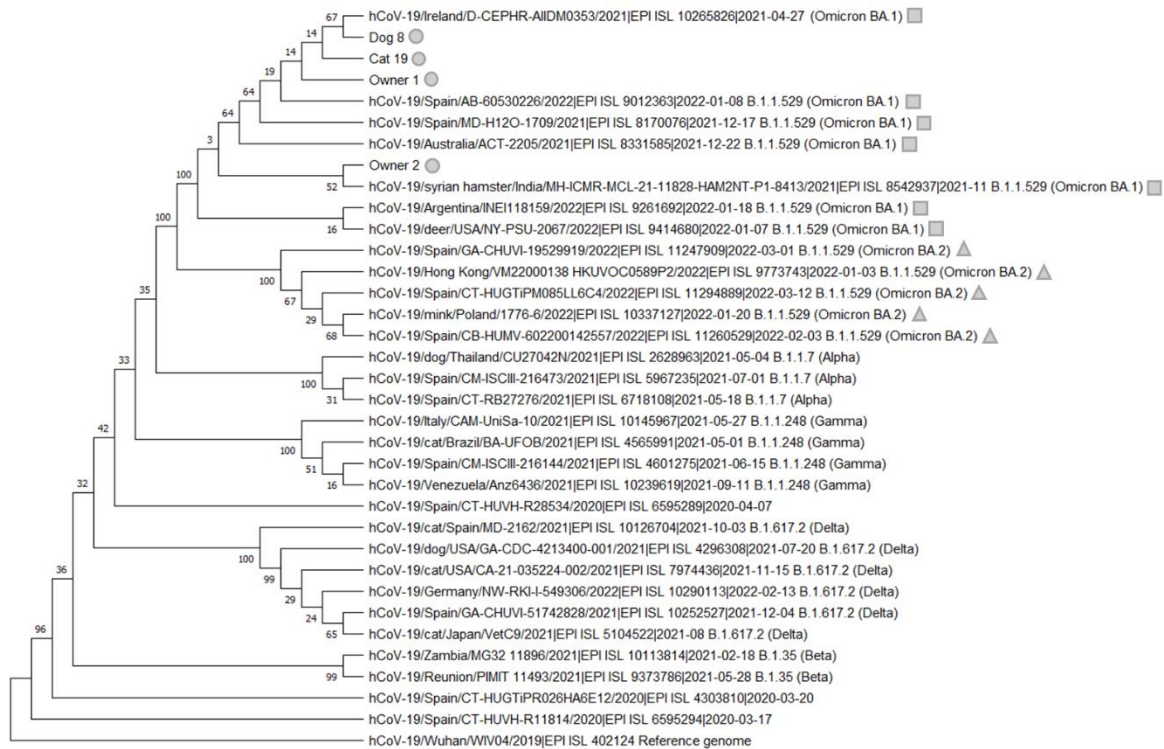
199 *Neutralizing antibodies detection by VNT*

200 Sera were collected from 15 animals (1 cat and 14 dogs), including Dog\_8 and Cat\_13 which  
 201 were also positive for RT-qPCR (15 and 20 days after RT-qPCR positive result,  
 202 respectively). Among the 15 serum samples, none of them presented neutralizing antibodies.

203 *Whole-genome sequencing and phylogenetic analysis*

204 The complete genome sequence of SARS-CoV-2 was obtained from the oropharyngeal swabs  
 205 from both Dog\_8 and Cat\_19 (GenBank accession numbers: ON115270 and ON115269;  
 206 GISAID accession ID: EPI\_ISL\_11580532 and EPI\_ISL\_11580576 ) as well as from

207 nasopharyngeal swabs from the owner of Dog\_8 (Owner\_1; GenBank accession numbers:  
208 ON115271; GISAID accession ID: EPI\_ISL\_11580604) and the owner of Cat\_26, 27 and 28  
209 (Owner\_2; GenBank accession number: ON115272; GISAID accession ID:  
210 EPI\_ISL\_11580636) since the remaining PCR-positive animals had too low viral RNA loads  
211 for effective sequencing.



213 Figure 1. Phylogenetic analysis of SARS-CoV-2 of the whole-genome sequences from  
214 Dog\_8, Cat\_19, Owner\_1, and Owner\_2 (grey circle), which were clustered with the SARS-  
215 CoV-2 B.1.1.529 (Omicron) and more specifically with lineage BA.1 (grey square). The  
216 lineage BA.2 is indicated with a grey triangle.

217 Analysis in the CoVsurver mutations app (GISAID) showed that the sequences presented  
218 several mutations having as a reference the hCoV-19/Wuhan/WIV04/2019 sequence. The  
219 mutations were 37 in the case of Dog\_8 and Cat\_19 (Table 2, Table 3). No variabilities were

220 observed at the nucleotide/amino acid level between the sequences from Dog\_8 and its owner  
 221 (Owner\_1).

222 Table 2. List of mutations displayed in the different regions of the genome of SARS-CoV-2  
 223 in the sequence obtained in this study of Dog\_8. NSP: Non-structural protein; E: envelope  
 224 protein; M: Membrane protein; N: Nucleocapsid protein.

Location in the genome	Mutations displayed
NSP3 (ORF1a)	K38R, P985S, V1069I, S1265del, L1266I, A1892T
NSP4 (ORF1a)	T492I
NSP5	P132H
NSP6 (ORF1a)	L105del, S106del, G107del, I189V
NSP12 (ORF 1b)	P323L
NSP14 (ORF 1b)	I42V
Spike	A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F
E	T9I
M	D3G, Q19E, A63T
N	P13L, E31del, R32del, S33del, R203K, G204R

225

226 Table 3. List of mutations displayed in the different regions of the genome of SARS-CoV-2  
 227 in the sequence obtained in this study of Cat\_19. NSP: Non-structural protein; NS3: Non-  
 228 structural protein 3; E: envelope protein; M: Membrane protein; NS7a: Accessory protein 7a;  
 229 N: Nucleocapsid protein.

230

Location in the genome	Mutations displayed
NSP3 (ORF1a)	K38R, P985S, V1069I, S1265del, L1266I, A1892T
NSP4 (ORF1a)	T492I
NSP5	P132H
NSP6 (ORF1a)	L105del, S106del, G107del, I189V
NSP12 (ORF 1b)	P323L
NSP14 (ORF 1b)	I42V
Spike	A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F
NS3	T14del, L15del
E	T9I
M	D3G, Q19E, A63T
NS7a	ins45EstopLN
N	P13L, E31del, R32del, S33del, R203K, G204R

231

## 232 **Discussion**

233 The SARS-CoV-2 B.1.529 (Omicron) variant, the last VOC detected, is nowadays highly  
234 extended around the world. Concretely in Spain, epidemiological data from the Omicron-  
235 associated wave has evidenced that the transmission rate of this variant is quite superior to  
236 other variants such as Beta or Delta. This fact has promoted the rapid spread of this variant,  
237 being dominant since November 2021 (He, Hong et al. 2021). One concern about this new  
238 variant is its potential transmission to other species, in which it could evolve and acquire new  
239 mutations that may be involved in higher virulence, among other fears. For this reason, it is  
240 necessary to evaluate its capability to infect susceptible species. In this sense, pets such as  
241 cats and dogs should be a major focus due to their close contact with humans.

242 In this study, we evidenced the detection of the Omicron SARS-CoV-2 variant in companion  
243 animals, demonstrating that pets are susceptible to the infection with this strain. However,  
244 according to the outcomes obtained by this work, there was a relatively low number of  
245 positive animals to RT-qPCR taken into account the characteristics of the study, which

246 involved an active sampling. In all the cases, owners assured high contact with their pets. In  
247 addition, the sampling was done at the best time for the detection of the disease (Shi, Wen et  
248 al. 2020) and only 10.13% of animals became infected, as well as no clinical signs were  
249 observed in any animal. These results contrast with previous reports in which the  
250 susceptibility of cats and dogs to other SARS-CoV-2 variants such as Alpha and Delta seems  
251 to be higher (Barroso-Arévalo, Sánchez-Morales et al. 2022; Barroso-Arevalo, Rivera et al.  
252 2021; Fernandez-Bastit, Rodon et al. 2021, Hamer, Ghai et al. 2021). Furthermore, in the  
253 case of animal naturally infected with these other variants, clinical signs were described  
254 (Segales, Puig et al. 2020, Ferasin, Fritz et al. 2021, Fernandez-Bastit, Rodon et al. 2021,  
255 Hamer, Ghai et al. 2021, Barroso-Arévalo, Sánchez-Morales et al. 2022) and higher viral  
256 loads were detected (Barroso-Arevalo, Rivera et al. 2021, Barroso-Arévalo, Sánchez-Morales  
257 et al. 2022). Another remarkable difference observed in animals infected with the Omicron  
258 variant is that viral isolation was not possible from any sample, due to the low viral load of  
259 all positive specimens. The fact that viral isolation was not possible could be due, in part, to  
260 the lower fusogenicity of this variant (Suzuki, Yamasoba et al. 2022) which may difficult the  
261 virus entry into the cell. By contrast, viral isolation from cat and dogs samples has been  
262 possible in the case of the original virus isolate (Hamer, Pauvolid-Correa et al. 2020,  
263 Barroso-Arevalo, Barneto et al. 2021) and other variants (Barroso-Arevalo, Rivera et al.  
264 2021). Neither was it possible to detect the presence of neutralizing antibodies in the RT-  
265 qPCR-positive animals in contrast to other SARS-CoV-2 seroprevalence studies in animals.  
266 This may be derived from the fact that virus replication may have been limited to the local  
267 level. In consequence, it is possible viral dissemination did not occur in the infected animals  
268 and what we have detected were remnants of viral RNA (Días et al., 2021). This could be  
269 explained by the fact that PCR-positive animals were only detected on one day of the 4 to 5  
270 consecutive days of sampling.

271 All these results may be explained by the fact that a higher affinity with the human cellular  
272 receptor has been reported in the case of the Omicron variant compared to other variants (He,  
273 Hong et al. 2021, Zou, Xia et al. 2021, Suzuki, Yamasoba et al. 2022). This could have led to  
274 the displacement of the binding between the animal cell and the virus, maybe due to specific  
275 variations in the ACE2 animal's receptor with respect to the human ACE2. This may be the  
276 reason for the variation of susceptibility in animals to this new variant compared to the  
277 previous ones. Further experimental research should be conducted to corroborate this  
278 hypothesis since non-experimental data on cats and dogs infected with the Omicron variant  
279 are available so far.

280 However, these results contrast with those of an experimental study carried out in mink  
281 (Virtanen, Aaltonen et al. 2022), in which high pathogenicity of the Omicron variant was  
282 observed, both at the level of symptoms and post mortem anatomopathological lesions. This  
283 higher susceptibility may be affected by the fact that mink-derived SARS-CoV-2 strains  
284 encode substitutions in areas of the genome crucial for ACE2 receptor binding that may  
285 enhance the binding of the spike protein to this receptor (Welkers, Han et al. 2021). It is,  
286 therefore, necessary to carry out studies on the pathogenicity of this variant in different  
287 animal species, as well as active surveillance to be able to early detection of new emerging  
288 variants.

289 Although so far there have been no publications on the presence of the Omicron variant in  
290 pets, it has been detected in wildlife, specifically in white-tailed deer (*Odocoileus*  
291 *virginianus*), which have been shown to be highly susceptible to the SARS-CoV-2 virus.  
292 Fortunately, despite their higher susceptibility, the risk of high contact with an infected  
293 human in this species is quite low, contrary to what is happening in the case of pets. These  
294 aspects highlight the importance of the investigation of these new variants both in urban and  
295 wild fauna (Vandegrift, Yon et al. 2022).

296 From what we have observed in this study, it appears that the Omicron variant is less virulent  
297 to pets than the previous variants as well as the original isolate. Although 10.13% of the  
298 animals analyzed in this field study tested positive for RT-qPCR, low viral loads were  
299 detected and none of the infected animals showed any symptomatology. This, together with  
300 our results previously obtained on VOCs in animals (Barroso-Arevalo, Rivera et al. 2021;  
301 Barroso-Arévalo, Sánchez-Morales et al. 2022) has demonstrated the great variability of  
302 pathogenicity and response of each animal species and the efficiency of our active  
303 surveillance system. This highlights the importance of conducting active surveillance in both  
304 pets living with COVID19 infected people and wildlife, in addition to genomic research to  
305 early detect infections with other variants or mutations associated with animal hosts. This  
306 also underlines the relevance of establishing a network of clinics and owners to be able to  
307 carry out active surveillance sampling.

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### 316 **Conflict of Interest**

317 The authors declare that the research was conducted in the absence of any commercial or  
318 financial relationships that could be construed as a potential conflict of interest.

319 **Author contributions**

320 SBA and JMSV designed the study. SBA and LSM performed the sampling and veterinary  
321 inspection. SBA and LSM performed laboratory analysis. LD and JMSV acquired the funds.  
322 SBA and LSM wrote the initial manuscript. LD, MPS, and JMSV reviewed the manuscript.  
323 All the authors have read and approved the final version of the manuscript.

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