A large-scale serological survey in pets from October 2020 through June 2021 in France shows significantly higher exposure to SARS-CoV-2 in cats

Matthieu Fritz[a], Eric Elguero[a,1], Pierre Becquart[a,1], Daphné de Riols de Fonclare[a], Déborah Garcia[a], Stephanie Beurlet [b], Solène Denolly [c], Bertrand Boson [c], Serge G. Rosolen [e, f], François-Loïc Cosset[c], Alexandra Briend-Marchal [b], Vincent Legros [c, d], Eric M. Leroy[a*]

[a] Maladies Infectieuses et Vecteurs, Ecologie, Génétique, Evolution et Contrôle (MIVEGEC), Univ. Montpellier, IRD, CNRS, 34394 Montpellier, France

[b] Laboratoire de Biologie vétérinaire VEBIO, Arcueil, France

[c] CIRI – Centre International de Recherche en Infectiologie, Team EVIR, Univ Lyon, Université Claude Bernard Lyon 1, Inserm, U1111, CNRS, UMR5308, ENS Lyon, Lyon, France.

[d] Université de Lyon, VetAgro Sup, Campus vétérinaire de Lyon, Marcy-l'Etoile, France

[e] Sorbonne Université, INSERM, CNRS, Institut de la Vision, Paris, France

[f] Clinique vétérinaire voltaire, Asnières, France

[1] Authors contributed equally

*Corresponding author:

Eric M.Leroy : UMR IRD-CNRS-UM, 911 avenue Agropolis, 34394 Montpellier, France

Tel : +33 467416107 ; E-mail address : eric.leroy@ird.fr

Keywords: SARS-CoV-2, Pets, Serology, Neutralization assay, Microsphere Immunoassay, One Health

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can infect many animals, including pets such as dogs and cats. Many studies have documented infection in companion animals by bio-molecular and serological methods. However, only a few have compared seroprevalence in cats and dogs from the general population, and these studies were limited by small sample sizes and collections over short periods. Our goal was to obtain a more accurate evaluation of seroprevalence in companion animals in France and to determine whether cats and dogs differ in their exposure to SARS-CoV-2. For this purpose, we conducted an extensive SARS-CoV-2 cross-sectional serological survey of 2036 cats and 3577 dogs sampled by veterinarians during medical examinations in clinics throughout France. Sampling was carried out from October 2020 through June 2021, a period encompassing the second and third waves of SARS-CoV-2 infections in humans in the country. Using a microsphere immunoassay targeting the receptor binding domain and trimeric spike protein, we found 7.1% seroprevalence in pets. In a subset of 308 seropositive samples, 26.3% had neutralizing antibodies. We found that cats were significantly more likely to test positive than dogs, with seropositivity rates of 9.3% and 5.9% in cats and dogs, respectively. Finally, data for both species showed that seroprevalence was lower in older animals and was not associated with the date of sampling or the sex of the animal. Our results show that cats are significantly more sensitive to SARS-CoV-2 than dogs, in line with experimental studies. Our large sample size provides for a reliable, statistically robust estimate of the frequency of infection of pets from their owners and offers strong support for the notion that cats are more sensitive to SARS-CoV-2 than dogs. Our findings emphasise the importance of a One-Health approach to the SARS-CoV-2 pandemic and raise the question of whether companion animals in close contact with humans should be vaccinated.

Introduction

46 Two months after the onset of SARS-CoV-2 circulation in humans, two dogs in Hong Kong were
47 reported to have naturally acquired the virus (1). Since then, many studies have reported viral RNA
48 and SARS-CoV-2 antibodies in dogs and cats—mostly belonging to COVID-19-infected owners (2-5).
49 Furthermore, several studies demonstrated that the risk of pets testing seropositive was higher in
50 COVID-19+ households than for pets from households of unknown status (6-10).

51 Definitive examples of pet-to-human transmission are scarce. A recent study from Thailand reported
52 a suspected case of SARS-CoV-2 transmission from a cat to a human (11), and dog-to-human
53 transmission has yet to be described. However, given that 200 million cats and dogs live in close
54 proximity to humans in Europe (12), there is ample opportunity for such transmission, and the
55 potential risks need to be carefully considered.

56 Several population-based serological studies have reported SARS-CoV-2 antibodies in dogs and cats.
57 In dogs, estimates of seroprevalence have ranged from 0% to 14.5% (6, 10, 13-21). While in cats,
58 estimates have ranged from 0% to 21.7% (6, 14, 15, 20, 22-25). For both species, seroprevalence was
59 highly dependent on the period of sampling (first, second wave *etc.*), the assay used (ELISA,
60 seroneutralization, etc.), and the country of sampling (China, Croatia, Germany, Italy, the
61 Netherlands, Poland, Portugal, Spain, Thailand, United-Kingdom, USA). Among these studies, five
62 directly compared cats and dogs. There is some experimental and epidemiological evidence
63 suggesting that cats are more susceptible to infection than dogs (6, 7, 15, 26, 27). However,
64 significant species differences have not always been observed in population-based studies (13, 17,
65 19). This is perhaps because of significant study limitations—a low number of enrolled animals, a
66 short sampling period, etc.—that have curtailed robust estimates of infection rate in pets with
67 enough statistical power to recognize differences in COVID-19 epidemiology.

68 Here we report estimates of the frequency of SARS-CoV-2 infection from 2036 cats and 3577 dogs
69 sampled at veterinary clinics from October 2020 through June 2021 throughout France—the largest
70 serological survey of SARS-CoV-2 infections in companion animals to date. The study allows for

71 robust estimates of pet infection rate and provides strong support for the hypothesis that species
72 differences in susceptibility observed in experimental studies translate into a significant increase in
73 infection rate in cats.

74 **Materials and Methods**

75 **Sampling**

76 The cross-sectional, nationwide sampling was possible thanks to a network of veterinary clinics
77 across France working with VEBIO. VEBIO is a veterinary diagnostic laboratory which performed all
78 categories of medical analyses, including infectious diseases, hematology, endocrinology, oncology …
79 (see more details in https://www.vebio.fr/). No inclusion and exclusion criteria were applied for the
80 selection of blood samples, except that they came only from veterinary clinics working with VEBIO.
81 VEBIO notified the veterinary clinics that following requested biomedical analyses, the remaining
82 serum could be used in a SARS-CoV-2 research project. No specific request for samples was
83 addressed to the vets. Thus, the SARS-CoV-2 analysis is based on samples collected during the regular
84 activities of the vets.

85 Blood samples were collected in dry/EDTA tubes from dogs and cats during routine healthcare visits
86 or for diagnostic purposes at veterinary clinics. After centrifugation, the serum/plasma was kept at
87 +4 °C until sent to VEBIO. Rapid and safe shipping practices were used to avoid contamination and
88 ensure samples reached VEBIO within 48 h. At the VEBIO facility, an aliquot was taken from the
89 sample to perform the requested biomedical analyses. Another aliquot was then stored at +4 °C until
90 sent to the MIVEGEC lab, Montpellier, where serological analyses were performed. Safe shipping
91 practices with an approved professional carrier were also used for shipment to the MIVEGEC lab.
92 Finally, the samples were stored at the MIVEGEC lab at −20 °C until testing (Figure 1). For shipping to
93 the CIRI lab, SARS-CoV-2-positive samples detected by MIA were transported by an approved
94 professional carrier at -20°C to ensure optimal safety conditions. Data (age, sex, clinical history, and

region localization, when available) from dogs and cats were provided anonymized by VEBIO to the MIVEGEC lab.

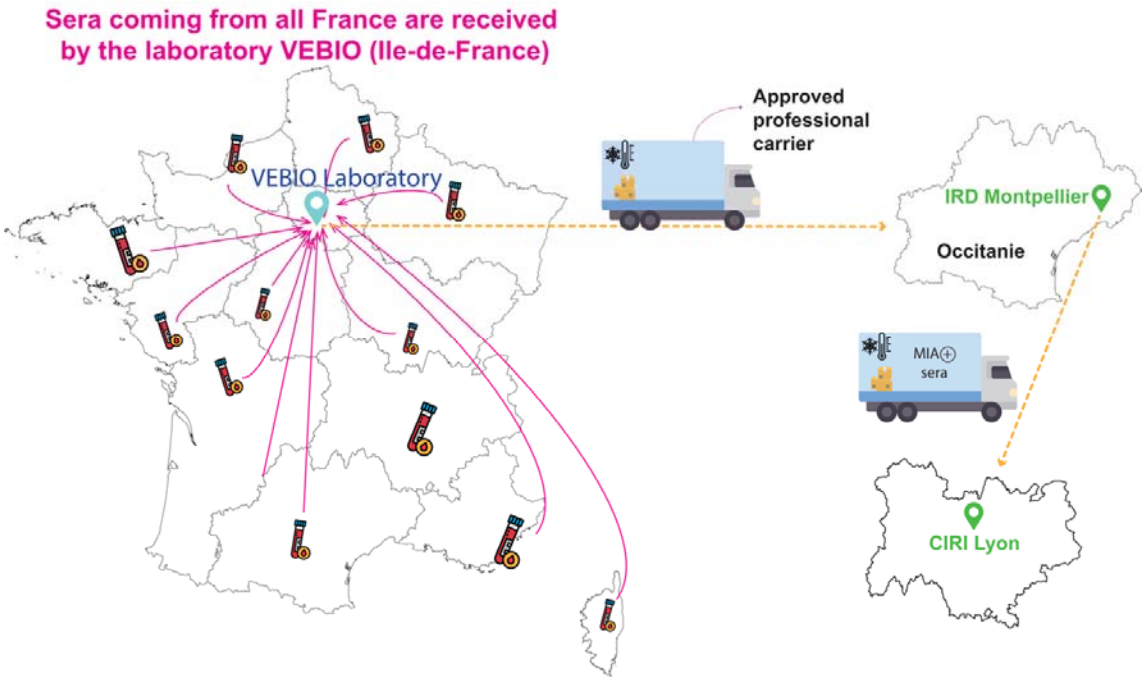


Figure 1. Logistics of sample collection and distribution. Sera collected during routine healthcare visits by veterinarians throughout France were first sent to VEBIO in Ile-de-France. Aliquots of the samples were then made and sent to the IRD in Montpellier (Hérault) via an approved carrier.

Ethics

According to the act governing the "use of live animals for scientific purposes" effective in France on 14 January 2022, ethical approval was not sought or required since all pets were sampled by a veterinarian during a health care visit. All applicable international and national guidelines for the care of pets were followed.

Microsphere Immunoassay (MIA)

Dog and cat serum samples were tested using a multiplex microsphere immunoassay (MIA). Ten µg of two recombinant SARS-CoV-2 antigens, receptor-binding domain (RBD) and trimeric spike (tri-S), both derived from the Whuhan-Hu-1 strain (The Native Antigen Company, Kidlington United-

110 Kingdom), were used to capture specific serum antibodies. Distinct MagPlex microsphere sets
111 (Luminex Corp, Austin, TX, USA) were respectively coupled to viral antigens using the amine coupling
112 kit (Bio-Rad Laboratories, Marnes-la-Coquette, France) according to the manufacturer's instructions.
113 Microsphere mixtures were successively incubated with serum samples (1:400), biotinylated protein
114 A and biotinylated protein G (4 µg/mL each) (Thermo Fisher Scientific, Illkirch, France), and
115 streptavidin-R-phycoerythrin (4 µg/mL) (Life technologies, Illkirch, France) on an orbital shaker and
116 protected from light. Measurements were performed using a Luminex 200 instrument (Luminex
117 Corp, Austin, TX, USA), and at least 100 events were read for each bead set. Binding events were
118 displayed as median fluorescence intensities (MFI). Specific seropositivity cut-off values for each
119 antigen were set at three standard deviations above the mean MFI of pre-pandemic serum from 53
120 dogs and 30 cats sampled before 2019. These samples were stored in biobanks at the IRD and
121 VetAgro Sup. MIA specificity was set for each antigen at 96.2% for dogs and 100% for cats based on
122 the pre-pandemic populations. MIA was first validated using sera from two COVID-19 PCR+ humans,
123 kindly provided by Meriadeg Ar Gouilh, and then with sera from SARS-CoV-2 PCR+ cats and dogs,
124 provided by several veterinarians.

125 Because of the excellent specificity observed for both antigens and to account for any isotypic
126 variability, an animal was deemed positive for SARS-CoV-2 antibodies following a positive result in at
127 least one of the two tests.

128 **Neutralization activity measurement**

129 An MLV-based pseudoparticle carrying a GFP reporter pseudotyped with SARS-CoV-2 spike protein
130 (Wuhan-Hu-1 strain) (SARS-CoV-2pp) was used to measure neutralizing antibody activity in cat and
131 dog sera. Each SARS-CoV-2-positive sample detected by MIA was processed according to a
132 neutralization procedure as previously described (28) . Briefly, for neutralization assays, a sample of
133 ~1×10^3 pseudoparticles was incubated with a 100-fold dilution of sera or control antibodies for
134 1 h at 37 °C before infection of Vero-E6R cells. At 72 h post-transduction, the percentage of GFP-

135 positive cells was determined by flow cytometry (at least 10 000 events recorded). The level of
136 infectivity is expressed as the percentage of GFP-positive cells and compared to cells infected with
137 SARS-CoV-2pp incubated without serum. As a control, the same procedure was done using RD114
138 pseudoparticles to identify sera with aspecific neutralization. Sera exhibiting more than 30% SARS-
139 CoV-2pp neutralization were considered positive. Pre-pandemic serum from France was used as a
140 negative control, and an anti-SARS-CoV-2 RBD antibody was used as a positive control.

141 **Statistical analyses**

142 Associations between SARS-CoV2 infection status (positive or negative) and the covariates region,
143 age, and sex were assessed using binomial (logistic) generalized linear models. The region was
144 defined by where the animal lived at the time of sampling. Age was that recorded by the
145 veterinarian, with variable precision, generally in months for young animals and whole years for
146 older animals. Its accuracy is unknown. The associated statistical tests were likelihood ratio tests. All
147 analyses were performed using R software (29).

148 **Results**

149 **Blood collection**

150 Blood samples from 2036 cats and 3577 dogs were collected during routine healthcare visits by
151 veterinarians from October 2020 through June 2021 (Table 1). Samples came from all 13 regions of
152 metropolitan France. Corsica was excluded due to too few samples. Almost half of the samples came
153 from Ile-de-France, the region including Paris, reflecting population density and proximity to the
154 Veterinary diagnostic laboratory (VEBIO), where all samples for biomedical analyses requested by the
155 veterinarians were handled (Materials and Methods). The number of samples received from other
156 regions largely depended on the number of veterinarians working with VEBIO in those regions (Figure
157 2). Unfortunately, we could not study the clinical history of the animals due to variability in how each
158 veterinarian reported this information.

	October 2020	November 2020	December 2023	January 2021	February 2021	March 2021	April 2021	May 2021	June 2021
Cats	49	256	275	291	225	296	305	166	173
Dogs	84	428	543	475	403	474	597	282	291
Total	133	684	818	766	628	770	902	448	464

Table 1 : numbers of samples collected by month and by species.

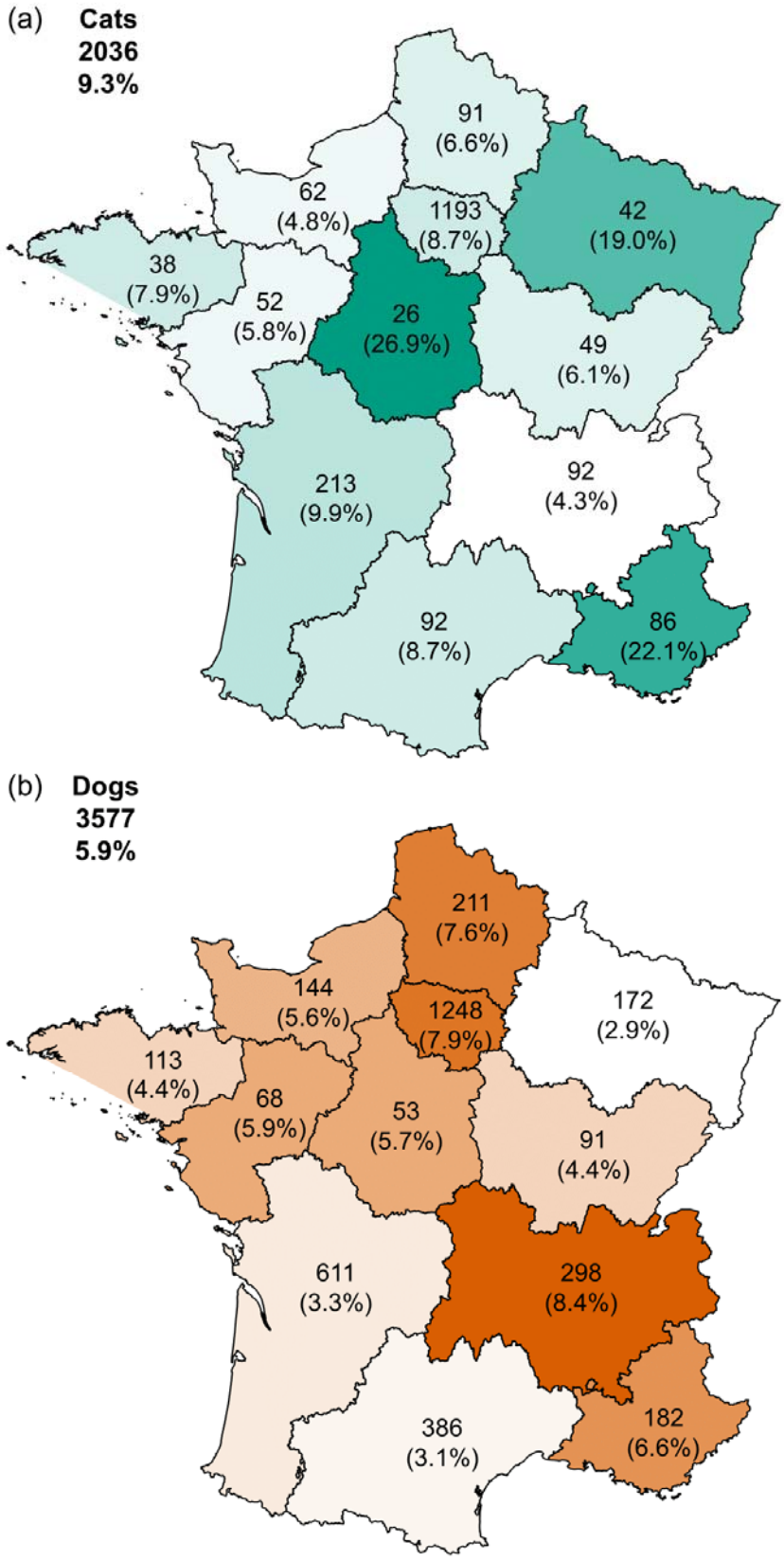

(a) Cats
2036
9.3%
91
(6.6%)
62
(4.8%)
1193
(8.7%)
42
(19.0%)
38
(7.9%)
52
(5.8%)
26
(26.9%)
49
(6.1%)
213
(9.9%)
92
(4.3%)
92
(8.7%)
86
(22.1%)
(b) Dogs
3577
5.9%
211
(7.6%)
144
(5.6%)
1248
(7.9%)
172
(2.9%)
113
(4.4%)
68
(5.9%)
53
(5.7%)
91
(4.4%)
611
(3.3%)
298
(8.4%)
386
(3.1%)
182
(6.6%)

Figure 2. (a). Map of France showing the number of SARS-CoV-2-positive cat sera per region. The total number of sera samples collected per region is indicated. Seroprevalence in each region is indicated as a percentage. Regions are shaded in green according to seroprevalence. The total number of sera samples and global seroprevalence for France is in the top left corner. **(b). Map of France showing the number of SARS-CoV-2-positive dog sera per region.** The total number of sera samples collected per region is indicated. Seroprevalence in each region is indicated as a percentage. Regions are shaded in orange according to seroprevalence. The total number of sera samples and global seroprevalence for France is in the top left corner.

Global seroprevalence

For the sera samples, 401 (7.1%) showed a positive result either against RBD, tri-S, or both (Supplementary Table 1). We next determined the presence of antibodies with neutralizing activity among the positive sera. To save time, we randomly tested approximately 75% (308) of positive sera samples. Seroneutralizing activity was detected in 81 (26.3%) of the 308 pet sera samples. Among these positive samples, 39 (48%) were positive for both RBD and tri-S, 39 (48%) were positive only for tri-s and 3 (4%) were only positive for RBD. Only the seroprevalence from MIA assays was analyzed in the remainder of the study.

Seroprevalence in cats and dogs

We observed that a significantly greater proportion of cats were positive (189/2036, 9.3%) than dogs (212/3577, 5.9%); OR = 1.62, 95% c.i. [1.32 - 1.99], P-value = 3.8e-06, Table 2. In addition, sera from MIA-positive cats were more likely to show neutralizing activity (49/144, 34%) than dogs (32/164, 19.5%); OR = 2.12, 95% c.i. [1.27 - 3.57], P-value = 0.0039). Species differences were not always significant within each region, likely due to reduced statistical power. However, when differences were significant, it was always the case that cats were more likely to be positive than dogs. (Table 2).

187 **Table 2.** Seroprevalence of IgG SARS-CoV-2 antibodies detected in blood samples from cats and dogs
188 collected in different French regions from October 2020 through June 2021. Data are presented as
189 No. positive, percentage (95 % exact binomial confidence intervals). Odds ratios were computed by
190 fitting binomial models region by region; an OR > 1 indicates cats were more likely to be positive than
191 dogs. In this analysis, individual data on pet age and sex were not considered, as age was not
192 available for all animals. P-values were computed by the likelihood ratio test.

193

Region	Cats	Cats seroprevalence		Dogs	Dogs seroprevalence		OR (95% c.i.)	P-value
Auvergne-Rhône-Alpes	4/92	4.3%	(1.2-10.8)	25/298	8.4%	(5.5-12.1)	0.50 (0.17 - 1.47)	0.17
Bourgogne-Franche-Comté	3/49	6.1%	(1.3-16.9)	4/91	4.4%	(1.2-10.9)	1.42 (0.30 - 6.61)	0.66
Bretagne	3/38	7.9%	(1.7-21.4)	5/113	4.4%	(1.5-10.0)	1.85 (0.42 - 8.14)	0.43
Centre-Val de Loire	7/26	26.9%	(11.6-47.8)	3/53	5.7%	(1.2-15.7)	6.14 (1.44 - 26.2)	0.0098
Grand Est	8/42	19.0%	(8.6-34.1)	5/172	2.9%	(1.0-6.7)	7.86 (2.42 - 25.5)	0.00057
Hauts-de-France	6/91	6.6%	(2.5-13.8)	16/211	7.6%	(4.4-12.0)	0.86 (0.33 - 2.27)	0.76
Île-de-France	104/1193	8.7%	(7.2-10.5)	98/1248	7.9%	(6.4-9.5)	1.12 (0.84 - 1.49)	0.44
Normandie	3/62	4.8%	(1.0-13.5)	8/144	5.6%	(2.4-10.7)	0.86 (0.22 - 3.37)	0.83
Nouvelle-Aquitaine	21/213	9.9%	(6.2-14.7)	20/611	3.3%	(2.0-5.0)	3.23 (1.72 - 6.09)	0.00037
Occitanie	8/92	8.7%	(3.8-16.4)	12/386	3.1%	(1.6-5.4)	2.97 (1.18 - 7.49)	0.028
Provence-Alpes-Côte d'Azur	19/86	22.1%	(13.9-32.3)	12/182	6.6%	(3.5-11.2)	4.02 (1.85 - 8.73)	0.00036
Pays de la Loire	3/52	5.8%	(1.2-15.9)	4/68	5.9%	(1.6-14.4)	0.98 (0.21 - 4.58)	0.98
Total	**189/2036**	**9.3%**	**(8.0 - 10.6)**	**212/3577**	**5.9%**	**(5.2 - 6.8)**	**1.62 (1.32 - 1.99)**	**$3.8x10^{-6}$**

Seroprevalence by sex

We found no significant sex differences in seropositivity rates, either for all animals (females: 6.9%; 163/2361; males 7.5%; 212/2842; p = 0.24) or among cats (females 9.4%; 78/827, males 9.8%; 99/1009, p = 0.68) and dogs (females: 5.5%; 85/1534, males: 6.2%; 113/1833; p = 0.27) tested separately (Table 3).

Table 3. Seroprevalence of IgG SARS-CoV-2 antibodies in blood samples from cats and dogs by sex from October 2020 through June 2021 Data are presented as No. positive, percentage (95 % exact binomial confidence intervals). Odds ratios > 1 indicate males are more likely to be positive than females and were computed by fitting binomial generalized linear models, with age as a controlling factor. P-values correspond to likelihood ratio tests.

Sex	Cats	Cat seroprevalence	Dogs	Dog seroprevalence	Cats + Dogs	Cats + Dogs seroprevalence
Female	78/827	9.4% (7.5-11.5)	85/1534	5.5% (4.4-6.8)	163/2361	6.9% (5.9-8.0)
Male	99/1009	9.8% (8.0-11.8)	113/1833	6.2% (5.1-7.4)	212/2842	7.5% (6.5-8.5)
Total	**177/1836**	**9.6% (8.3-11.1)**	**198/3367**	**5.9% (5.1-6.7)**	**375/5203**	**7.2% (6.5-7.9)**
OR	1.08 (0.75 - 1.54)		1.20 (0.87 - 1.67)		1.15 (0.91 - 1.47)	
P-value	0.68		0.27		0.24	

Seroprevalence by age

Age was reported for 1657 cats (range: 0.2 – 22yr) and 2781 dogs (range: 0.1 – 18.5yr). Among cats, 18.4% aged [0-3] years, 10.4% aged]3-9] years, and 6.4% aged over 9 years tested positive. Among dogs, 8.8% aged [0-3] years, 5.8% aged]3-9] years, and 5.1% aged over 9 years tested positive. Using a binomial model with age entered as a continuous variable, we observed a significant decrease in seroprevalence with age in cats (OR for a one-year increase in age = 0.91, 95% c.i. [0.88 -

0.94], p-value = 3.7e-08) and dogs (OR = 0.95, 95% c.i. [0.92 - 0.99], p-value = 0.016) (Figure 3) (Supplementary Table 2).

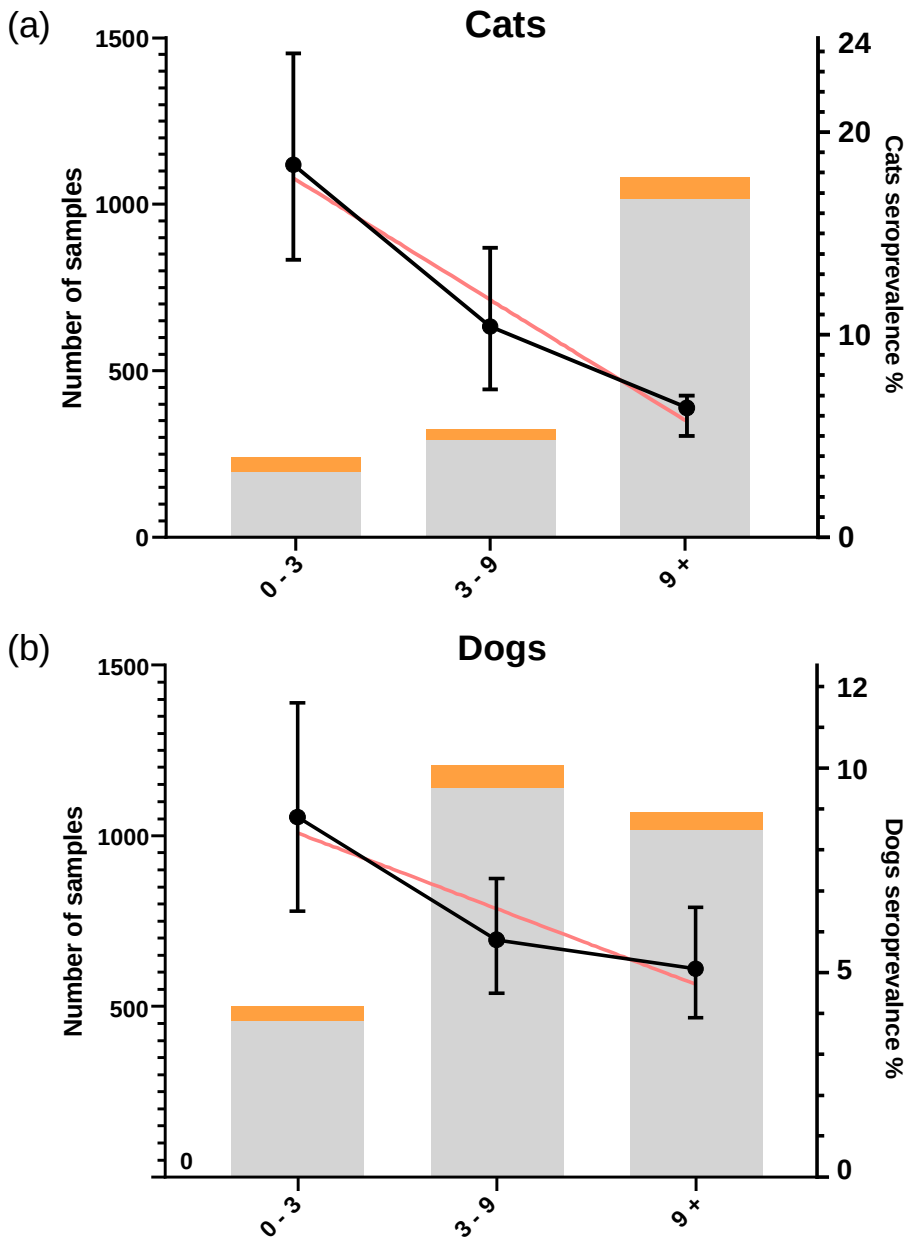


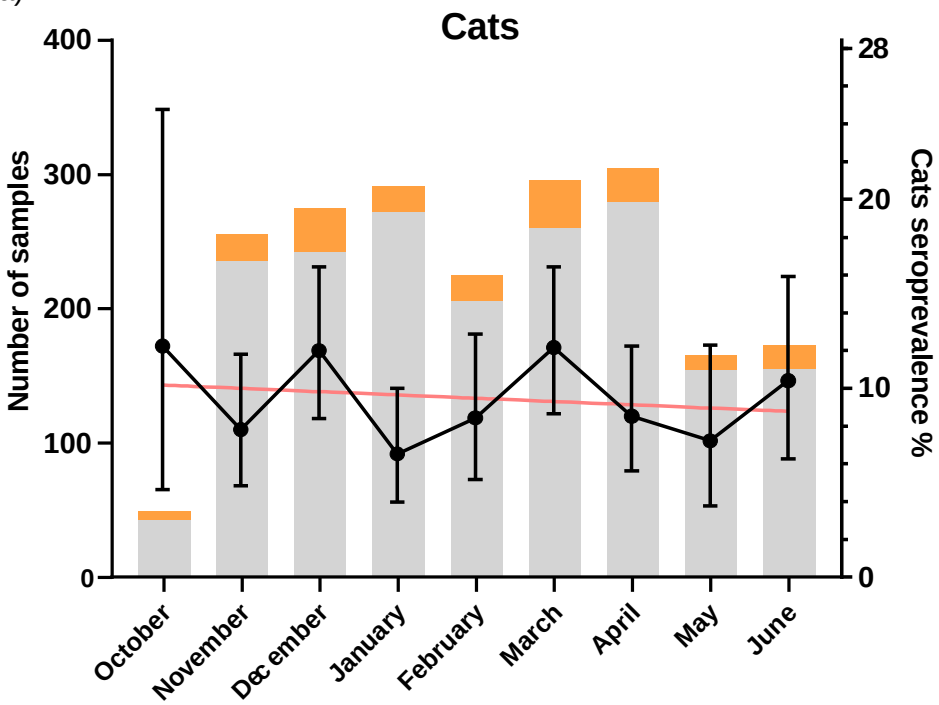
Figure 3. (a). The number of cat blood samples tested by age group for anti-SARS-CoV-2 antibodies by MIA from October 2020 through June 2021. Samples testing negative are shaded grey, and seropositive samples are in orange. Seroprevalence is represented by black dots, with 95 % binomial confidence interval. The red line represents the linear regression. **(b). The number of dog blood**

222 **samples tested by age group for anti-SARS-CoV-2 antibodies by MIA from October 2020 through**
223 **June 2021.** Samples testing negative are shaded grey, and seropositive samples are in orange.
224 Seroprevalence is represented by black dots, with 95 % binomial confidence intervals. The red line
225 represents the linear regression.
226

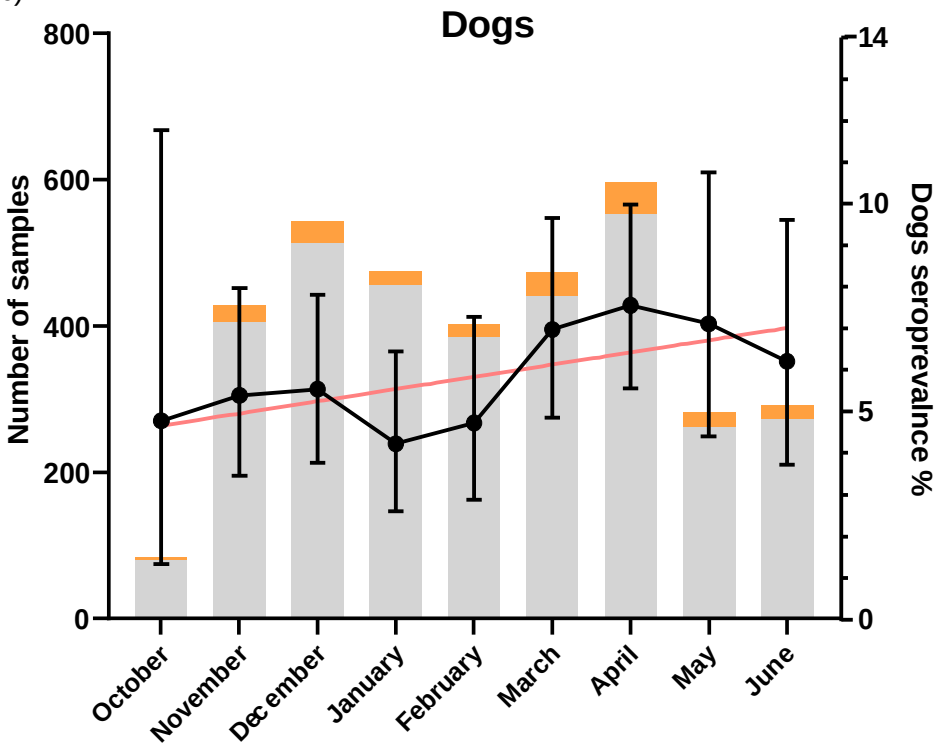
227 **Seroprevalence over time**

228 We next examined whether seroprevalence was associated with the time of sampling. For this
229 analysis, we selected animals at least one year old at the date of sampling (Figure 4). Seroprevalence
230 was not associated with the time of sampling for cats: p-value = 0.41. However, seroprevalence
231 among dogs increased over the 9 months of the study (OR = 3.47, 95% c.i. [1.47 - 8.23], p-value =
232 0.0045).
233

(a)


Cats
Number of samples
Cats seroprevalence %
400
300
200
100
0
28
20
10
0
October
November
December
January
February
March
April
May
June

(b)


Dogs
Number of samples
Dogs seroprevalnce %
800
600
400
200
0
14
10
5
0
October
November
December
January
February
March
April
May
June

Figure

4. (a). The number of cat blood samples tested each month for anti-SARS-CoV-2 antibodies by MIA from October 2020 through June 2021. Samples testing negative are shaded grey, and seropositive samples are in orange. Seroprevalence is represented by black dots, with 95 % binomial confidence interval. The red line represents the linear regression. **(b). The number of dog blood samples tested each month for anti-SARS-CoV-2 antibodies by MIA from October 2020 through June 2021.** Samples testing negative are shaded grey, and seropositive samples are in orange. Seroprevalence is represented by black dots, with 95 % binomial confidence interval. The red line represents the linear regression. Notice that in this figure, dates have been pooled by calendar month for illustrative purposes but that in the statistical analysis exact dates were used. Likewise, the regression lines are similarly illustrative as the statistical tests were based on logistic (binomial) regression.

Discussion

This study reports a large-scale serological survey of pet (cats and dogs) to detect anti-SARS-CoV-2 IgG antibodies. The samples were collected in metropolitan France from October 2020 through June 2021, a period including peaks of the second and third waves of SARS-CoV-2 infections in France. From a sample of 5613 pets, we reported a seroprevalence of anti-SARS-CoV-2 antibodies of 7.1%. We observed that only a small percentage of samples (48%) were positive for both tri-s and RBD, indicating that the RBD assay may be less sensitive than the tri-s assay. This may be explained by the fact that the full trimeric spike antigen may bind a broader range of antibodies than the receptor-binding domain, which includes only a small part of the spike protein. We found neutralizing antibody activity in the sera of only 26% of seropositive pets. Previous studies have shown that some pets do not develop neutralizing antibodies (5, 30). Cats were more likely to produce neutralizing antibodies than dogs, which is likely associated with a more prolonged and intense immune stimulation in cats. In humans, disease severity is positively correlated with neutralizing antibody levels(31).

260 In cats, we found a higher seroprevalence (9.3%) than previously observed in other European
261 countries, which ranged from 0% to 6.4% (13, 17-19, 21-24). However, most of these studies were
262 done before the second wave, during a period of relatively lower viral circulation than our sampling
263 period. In addition, most of these studies used a seroneutralisation assay.

264 In dogs, the observed seroprevalence (5.9%) is in accord with a previous study in France showing a
265 prevalence of 4.8% in companion and military working dogs sampled between February 2020 and
266 February 2021 (16). Other studies looking for SARS-CoV-2 antibodies in dogs have reported
267 seroprevalences ranging from 0% to 14.5% (10, 13, 17-19, 21).

268 Importantly, we observed a significantly higher seroprevalence of anti-SARS-CoV-2 antibodies in cats
269 than in dogs (p = 4.2e-08). The statistical significance of this difference varied among regions, likely
270 due to the reduced power and perhaps some unintended sampling bias by veterinarians. For
271 example, the smallest sample size was in the Bourgogne-Franche-Comté region, where we observed
272 no significant difference between dogs and cats. Furthermore, for a region like Ile-de-France, where
273 people live mostly in apartments, we can also hypothesize that dogs live in closer contact with
274 owners than in the rest of France. Previous studies with fewer samples have either found no
275 significant difference between species (8, 9, 13, 17, 19, 32) or that cats have significantly higher
276 seroprevalence than dogs (3, 6, 7).

277 Our study of a very large population of dogs and cats in natural conditions provides some evidence
278 that cats are more susceptible to SARS-CoV-2 infection than dogs, at least during the time frame of
279 our sampling period. Potential causes of species differences in susceptibility between cats and dogs
280 are numerous, but likely include a variety of biological and behavioural factors, as well as differences
281 in exposure. Intererestingly, ACE-2 shows greater sequence similarity between cat and human
282 orthologs than observed between dogs and humans (33). The absence of data such as the pet
283 lifestyle (Indoor/Outdoor), or the frequency and nature of contacts with humans and other animals

284 restricts our ability to identify a potential cause of the observed difference. In previous studies, most
285 infected pets were epidemiologically linked to humans who had tested positive for COVID-19 (34).

286 We did not observe significant sex differences in seroprevalence in either species (p = 0.45). Our
287 findings are consistent with most previous studies also reporting an absence of sex differences in
288 dogs and cats (6, 17, 32, 35). A smaller study of 188 dogs and 61 cats found higher seropositivity in
289 male dogs and an absence of a sex difference in cats (9). Another study found that male dogs
290 sampled from the general population were more likely to test positive than females, but this
291 difference was not observed in dogs from COVID-19+ households (10). There is little evidence of a
292 significant sex difference in susceptibility in humans. However, men are more likely to be affected by
293 severe forms of COVID than women for a variety of reasons (36).

294 In terms of age, we observed a higher seroprevalence among younger animals (between 0-3 years)
295 for both species that then decreased with age. A study of dogs sampled from the general population
296 found seroprevalence was highest in animals aged 5-6 years and that in COVID-19+ households,
297 seroprevalence peaked in slightly younger dogs, aged between one and five years (10). Other studies
298 have reported no significant associations with age in cats and dogs (6, 17). An experimental study in
299 cats found that juveniles appear more vulnerable than subadults (27). The decreasing seroprevalence
300 we observed with age could also arise from age-dependent behavioural changes. For example, young
301 animals (< 3 years old) are more active and curious and may be in greater contact with their owners
302 than older animals that prefer to remain quieter. The decrease could also reflect immunosenescence
303 in older animals, as observed in humans.

304 Interestingly, we observed a slight increase in seroprevalence in dogs during the study's nine months
305 of sampling, a trend not observed among cats. We expected an increase because antibodies have a
306 longer persistence in the organism than viral RNA; thus, animals sampled at later dates would
307 represent an accumulation of cases. The absence of a positive association between seroprevalence
308 and the time of sampling in cats has been reported in two other studies in Europe, but conclusions

309 were limited by the small number of samples collected over just a few months (24, 37). The absence
310 of an association in cats suggests a limited persistence of antibodies in cats than dogs. Few studies
311 have investigated variation in the persistence of antibodies in animals. For example, a study carried
312 out on seven dogs and two cats infected in natural conditions showed persistence of neutralizing
313 antibodies up to 10 months after infection in four of the dogs and the two cats, but also that
314 persistence was markedly reduced in two of the dogs after three months (38). Moreover, a study of
315 two cats found that neutralizing antibodies had disappeared by 110 days (25). Based on these data,
316 one possible reason for the lack of increase in seroprevalence during our study period could be a
317 progressive seroreversion of infected cats that is equally compensated by the number of new
318 infections, i.e. seroconversion. If so, this would mean that the observed seroprevalence is not an
319 accurate reflection of the total number of infections, at least in cats, during the whole epidemic.
320 Instead, seroprevalence provides a snapshot if infections acquired during a time period that remains
321 to be defined by longitudinal serological studies of cats and dogs. This also suggests that the
322 seroprevalence observed in our study may underestimate the actual proportion of cats infected
323 during the entirety of the epidemic.

324 Human-to-pet transmission may promote viral adaptation facilitating re-infection with novel viral
325 strains in humans (39). While one case of infection from cat to human has recently been reported,
326 the large number of pet cats and their frequent close interaction with humans provides ample
327 opportunity. This possibility raises the question of a vaccination strategy for animals susceptible to
328 SARS-CoV-2 infection. While pets do not currently seem to play a role in the ongoing pandemic, our
329 results emphasize the magnitude of SARS-CoV-2 infection in pets is not trivial. Combined with the
330 size of domestic cat and dog populations and the close contact with their human companions, our
331 results highlight the importance of collecting more data on SARS-CoV-2 transmissibility and
332 pathogenicity in companion animals, especially with the emergence of new variants. Also, when a
333 SARS-CoV-2 infection is suspected in a pet, we suggest collecting a sample for RT-qPCR confirmation
334 of infection, followed by whole-genome sequencing to identify new mutations, particularly in

antigenic sites targeted by the immune system. Finally, similar public health recommendations applied to humans should also be implemented for animals to prevent human-to-animal transmission, such as not having contact with animals when a household member is COVID-19 positive.

Acknowledgements: We are grateful to the pet owners for giving us permission to sample their pets. We thank the veterinarians that helped us with sampling. We thank Simon Thierry and Trung Thanh Nguyen for technical support and assistance. We also thank Kurt McKean (https://octopusediting.com/) for the English editing of the manuscript.

Funding: The study was funded by the French National Agency for Research (ANR-RA-COVID-19; Geographical and temporal serological investigation of companion animal infection with SARS-CoV-2 during the second wave of COVID-19 in France, CoVet). This study received financial support through a research grant from IDEXLYON project of Université de Lyon as part of the "Programme Investissements d'Avenir" (ANR-16-IDEX-0005). The study was funded by OIE through the European Union "EBO-SURSY" project.

Conflicts of Interest: The authors declare no conflicts of interest.

Institutional Review Board Statement: According to the act of "use of live animals for scientific purposes" effective in France on 14 January 2022, ethical approval was not sought or required since all pets were sampled by a veterinarian during a health care visit. All applicable international and national guidelines for the care of pets were followed.

Informed Consent Statement: Not applicable.

Data Availability Statement: Raw data and scripts are available on zenodo: https://doi.org/10.5281/zenodo.7646559

Author Contributions:

M.F., P.B., S.G.R., A.B.-M., V.L., and E.M.L. conceived and designed the study.

M.F., E.E., D.d.R.d.F., D.G., S.D., B.B., and V.L designed and performed the experiments.

All authors analyzed the data and interpreted and discussed the results.

M.F. and E.L. wrote the manuscript with input from all authors.

All authors have read and agreed to the published version of the manuscript.

References

1. Sit THC, Brackman CJ, Ip SM, Tam KWS, Law PYT, To EMW, et al. Infection of dogs with SARS-CoV-2. Nature. 2020;586(7831):776-8.
2. Barrs V, Peiris M, Tam KWS, Law PYT, Brackman C, To EMW, et al. SARS-CoV-2 in Quarantined Domestic Cats from COVID-19 Households or Close Contacts, Hong Kong, China. Emerging Infectious Disease journal. 2020;26(12):3071.
3. Hamer SA, Pauvolid-Corrêa A, Zecca IB, Davila E, Auckland LD, Roundy CM, et al. SARS-CoV-2 Infections and Viral Isolations among Serially Tested Cats and Dogs in Households with Infected Owners in Texas, USA. Viruses. 2021;13(5):938.
4. Jairak W, Charoenkul K, Chamsai E, Udom K, Chaiyawong S, Bunpapong N, et al. First cases of SARS-CoV-2 infection in dogs and cats in Thailand. Transboundary and emerging diseases. 2021.
5. Krafft E, Denolly S, Boson B, Angelloz-Pessey S, Levaltier S, Nesi N, et al. Report of One-Year Prospective Surveillance of SARS-CoV-2 in Dogs and Cats in France with Various Exposure Risks: Confirmation of a Low Prevalence of Shedding, Detection and Complete Sequencing of an Alpha Variant in a Cat. Viruses. 2021;13(9):1759.
6. Barroso R, Vieira-Pires A, Antunes A, Fidalgo-Carvalho I. Susceptibility of Pets to SARS-CoV-2 Infection: Lessons from a Seroepidemiologic Survey of Cats and Dogs in Portugal. Microorganisms. 2022;10(2):345.
7. Colitti B, Bertolotti L, Mannelli A, Ferrara G, Vercelli A, Grassi A, et al. Cross-Sectional Serosurvey of Companion Animals Housed with SARS-CoV-2–Infected Owners, Italy. Emerging Infectious Disease journal. 2021;27(7).
8. Fritz M, Rosolen B, Krafft E, Becquart P, Elguero E, Vratskikh O, et al. High prevalence of SARS-CoV-2 antibodies in pets from COVID-19+ households. One Health. 2021;11:100192.
9. 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. Nature Communications. 2020;11(1):6231.
10. Stevanovic V, Tabain I, Vilibic-Cavlek T, Mauric Maljkovic M, Benvin I, Hruskar Z, et al. The Emergence of SARS-CoV-2 within the Dog Population in Croatia: Host Factors and Clinical Outcome. Viruses. 2021;13(8):1430.
11. Sila T, Sunghan J, Laochareonsuk W, Surasombatpattana S, Kongkamol C, Ingviya T, et al. Suspected Cat-to-Human Transmission of SARS-CoV-2, Thailand, July-September 2021. Emerg Infect Dis. 2022;28(7):1485-8.
12. The European Pet Food Industry. FACTS & FIGURES 2020 European Overview. 2020.
13. Barroso-Arévalo S, Barneto A, Ramos ÁM, Rivera B, Sánchez R, Sánchez-Morales L, et al. Large-scale study on virological and serological prevalence of SARS-CoV-2 in cats and dogs in Spain. Transboundary and emerging diseases. 2021.
14. Barua S, Hoque M, Adekanmbi F, Kelly P, Jenkins-Moore M, Torchetti MK, et al. Antibodies to SARS-CoV-2 in dogs and cats, USA. Emerging Microbes & Infections. 2021:1-20.
15. 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.

402 16. Laidoudi Y, Sereme Y, Medkour H, Watier-Grillot S, Scandola P, Ginesta J, et al. SARS-CoV-2
403 antibodies seroprevalence in dogs from France using ELISA and an automated western blotting assay.
404 One Health. 2021;13:100293.
405 17. Pomorska-Mól M, Turlewicz-Podbielska H, Gogulski M, Ruszkowski JJ, Kubiak M, Kuriga A, et
406 al. A cross-sectional retrospective study of SARS-CoV-2 seroprevalence in domestic cats, dogs and
407 rabbits in Poland. BMC Veterinary Research. 2021;17(1):322.
408 18. Smith SL, Anderson ER, Cansado-Utrilla C, Prince T, Farrell S, Brant B, et al. SARS-CoV-2
409 neutralising antibodies in dogs and cats in the United Kingdom. Current Research in Virological
410 Science. 2021;2:100011.
411 19. Stevanovic V, Vilibic-Cavlek T, Tabain I, Benvin I, Kovac S, Hruskar Z, et al. Seroprevalence of
412 SARS-CoV-2 infection among pet animals in Croatia and potential public health impact.
413 Transboundary and emerging diseases. 2020;n/a(n/a).
414 20. Udom K, Jairak W, Chamsai E, Charoenkul K, Boonyapisitsopa S, Bunpapong N, et al.
415 Serological survey of antibodies against SARS-CoV-2 in dogs and cats, Thailand. Transboundary and
416 emerging diseases. 2021.
417 21. Zhao S, Schuurman N, Li W, Wang C, Smit LAM, Broens E, et al. Serologic Screening of Severe
418 Acute Respiratory Syndrome Coronavirus 2 Infection in Cats and Dogs during First Coronavirus
419 Disease Wave, the Netherlands. Emerging Infectious Disease journal. 2021;27(5):1362.
420 22. Michelitsch A, Hoffmann D, Wernike K, Beer M. Occurrence of Antibodies against SARS-CoV-2
421 in the Domestic Cat Population of Germany. Vaccines (Basel). 2020;8(4).
422 23. Michelitsch A, Schön J, Hoffmann D, Beer M, Wernike K. The Second Wave of SARS-CoV-2
423 Circulation—Antibody Detection in the Domestic Cat Population in Germany. Viruses.
424 2021;13(6):1009.
425 24. Schulz C, Martina B, Mirolo M, Müller E, Klein R, Volk H, et al. SARS-CoV-2–Specific Antibodies
426 in Domestic Cats during First COVID-19 Wave, Europe. Emerging Infectious Disease journal.
427 2021;27(12).
428 25. Zhang Q, Zhang H, Gao J, Huang K, Yang Y, Hui X, et al. A serological survey of SARS-CoV-2 in
429 cat in Wuhan. Emerg Microbes Infect. 2020;9(1):2013-9.
430 26. Bosco-Lauth AM, Hartwig AE, Porter SM, Gordy PW, Nehring M, Byas AD, et al. Experimental
431 infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to
432 reexposure in cats. Proceedings of the National Academy of Sciences. 2020;117(42):26382-8.
433 27. Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, et al. Susceptibility of ferrets, cats, dogs,
434 and other domesticated animals to SARS-coronavirus 2. Science. 2020;368(6494):1016-20.
435 28. Legros V, Denolly S, Vogrig M, Boson B, Siret E, Rigaill J, et al. A longitudinal study of SARS-
436 CoV-2-infected patients reveals a high correlation between neutralizing antibodies and COVID-19
437 severity. Cellular & Molecular Immunology. 2021;18(2):318-27.
438 29. R Development Core Team. R: A language and environment for statistical computing. Vienna,
439 Austria: R Foundation for Statistical Computing; 2018.
440 30. Ferasin L, Fritz M, Ferasin H, Becquart P, Corbet S, Ar Gouilh M, et al. Infection with SARS-
441 CoV-2 variant B.1.1.7 detected in a group of dogs and cats with suspected myocarditis. Vet Rec.
442 2021;189(9):e944.
443 31. Garcia-Beltran WF, Lam EC, Astudillo MG, Yang D, Miller TE, Feldman J, et al. COVID-19-
444 neutralizing antibodies predict disease severity and survival. Cell. 2021;184(2):476-88.e11.
445 32. Calvet GA, Pereira SA, Ogrzewalska M, Pauvolid-Corrêa A, Resende PC, Tassinari WdS, et al.
446 Investigation of SARS-CoV-2 infection in dogs and cats of humans diagnosed with COVID-19 in Rio de
447 Janeiro, Brazil. PLOS ONE. 2021;16(4):e0250853.
448 33. Damas J, Hughes Graham M, Keough Kathleen C, Painter Corrie A, Persky Nicole S, Corbo M,
449 et al. Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in
450 vertebrates. Proceedings of the National Academy of Sciences. 2020;117(36):22311-22.
451 34. Maurin M, Fenollar F, Mediannikov O, Davoust B, Devaux C, Raoult D. Current Status of
452 Putative Animal Sources of SARS-CoV-2 Infection in Humans: Wildlife, Domestic Animals and Pets.
453 Microorganisms. 2021;9(4).

35. Jairak W, Charoenkul K, Chamsai E, Udom K, Chaiyawong S, Hangsawek A, et al. Survey of SARS-CoV-2 in dogs and cats in high-risk areas during the second wave of COVID-19 outbreak, Thailand. Zoonoses and Public Health. 2022.

36. Bechmann N, Barthel A, Schedl A, Herzig S, Varga Z, Gebhard C, et al. Sexual dimorphism in COVID-19: potential clinical and public health implications. The Lancet Diabetes & Endocrinology. 2022.

37. Adler JM, Weber C, Wernike K, Michelitsch A, Friedrich K, Trimpert J, et al. Prevalence of anti-severe acute respiratory syndrome coronavirus 2 antibodies in cats in Germany and other European countries in the early phase of the coronavirus disease-19 pandemic. Zoonoses and Public Health.n/a(n/a).

38. Decaro N, Grassi A, Lorusso E, Patterson EI, Lorusso A, Desario C, et al. Long-term persistence of neutralizing SARS-CoV-2 antibodies in pets. Transboundary and emerging diseases. 2021.

39. Bashor L, Gagne RB, Bosco-Lauth AM, Bowen RA, Stenglein M, VandeWoude S. SARS-CoV-2 evolution in animals suggests mechanisms for rapid variant selection. Proceedings of the National Academy of Sciences. 2021;118(44):e2105253118.