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

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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 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 (Sit et al., 2020). Since then, many studies have
48 reported viral RNA and SARS-CoV-2 antibodies in dogs and cats—mostly belonging to COVID-19-
49 infected owners (Barrs et al., 2020; Hamer et al., 2021; Jairak et al., 2021; Krafft et al., 2021).
50 Furthermore, several studies demonstrated that the risk of pets testing seropositive was higher in
51 COVID-19+ households than for pets from households of unknown status (Patterson et al., 2020;
52 Colitti et al., 2021; Fritz et al., 2021; Stevanovic et al., 2021; Barroso et al., 2022).

53 Definitive examples of pet-to-human transmission are scarce. A recent study from Thailand reported
54 a suspected case of SARS-CoV-2 transmission from a cat to a human (Sila et al., 2022), and dog-to-
55 human transmission has yet to be described. However, given that 200 million cats and dogs live in
56 close proximity to humans in Europe (The European Pet Food Industry, 2020), there is ample
57 opportunity for such transmission, and the potential risks need to be carefully considered.

58 Several population-based serological studies have reported SARS-CoV-2 antibodies in dogs and cats.
59 In dogs, estimates of seroprevalence have ranged from 0% to 14.5% (Stevanovic et al., 2020;
60 Barroso-Arévalo et al., 2021; Barua et al., 2021; Dileepan et al., 2021; Laidoudi et al., 2021;
61 Pomorska-Mól et al., 2021; Smith et al., 2021; Stevanovic et al., 2021; Udom et al., 2021; Zhao et al.,
62 2021; Barroso et al., 2022). While in cats, estimates have ranged from 0% to 21.7% (Michelitsch et
63 al., 2020; Zhang et al., 2020; Barua et al., 2021; Dileepan et al., 2021; Michelitsch et al., 2021; Schulz
64 et al., 2021; Udom et al., 2021; Barroso et al., 2022). For both species, seroprevalence was highly
65 dependent on the period of sampling (first, second wave *etc.*), the assay used (ELISA,
66 seroneutralization, etc.), and the country of sampling (China, Croatia, Germany, Italy, the
67 Netherlands, Poland, Portugal, Spain, Thailand, United-Kingdom, USA). Among these studies, five
68 directly compared cats and dogs. There is some experimental and epidemiological evidence
69 suggesting that cats are more susceptible to infection than dogs (Bosco-Lauth et al., 2020; Shi et al.,
70 2020; Colitti et al., 2021; Dileepan et al., 2021; Barroso et al., 2022). However, significant species

71 differences have not always been observed in population-based studies (Stevanovic et al., 2020;
72 Barroso-Arévalo et al., 2021; Pomorska-Mól et al., 2021). This is perhaps because of significant study
73 limitations—a low number of enrolled animals, a short sampling period, etc.—that have curtailed
74 robust estimates of infection frequency in pets with enough statistical power to recognize
75 differences in COVID-19 epidemiology.

76 Here we report estimates of the frequency of SARS-CoV-2 infection from 2036 cats and 3577 dogs
77 sampled at veterinary clinics from October 2020 through June 2021 throughout France—the largest
78 serological survey of SARS-CoV-2 infections in companion animals to date. The study allows for
79 robust estimates of pet infection frequency and provides strong support for the hypothesis that
80 species differences in susceptibility observed in experimental studies translate into a significant
81 increase in infection frequency in cats.

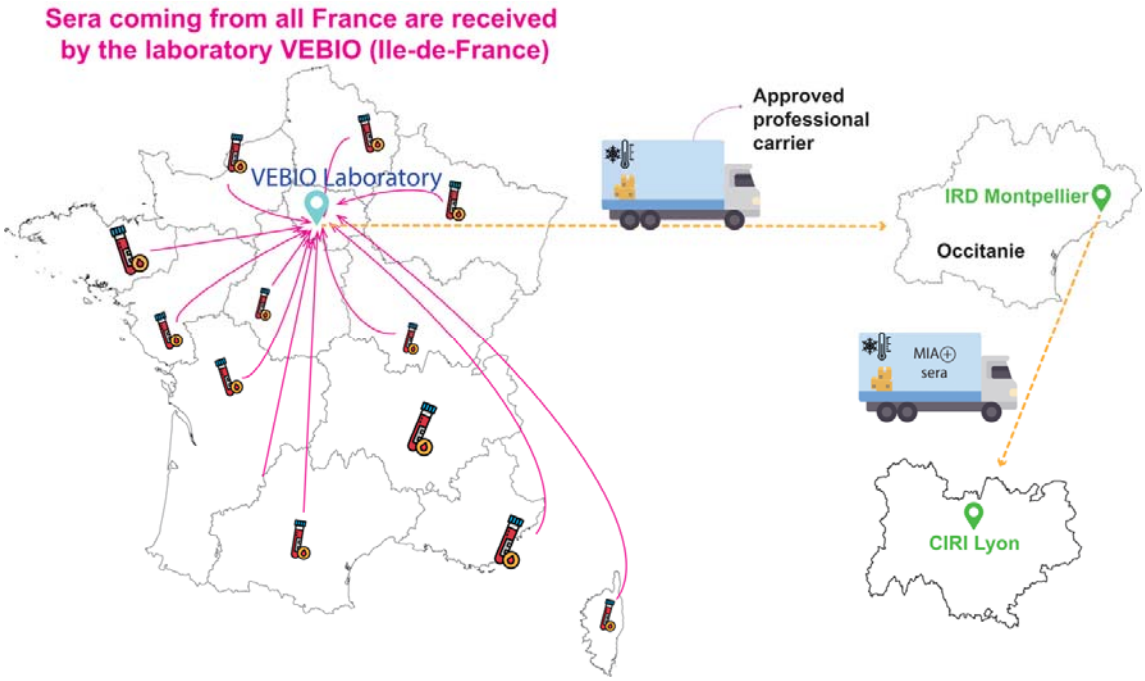
82 **Materials and Methods**

83 **Sampling**

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

93 Blood samples were collected in dry/EDTA tubes from dogs and cats during routine healthcare visits
94 or for diagnostic purposes at veterinary clinics. After centrifugation, the serum/plasma was kept at
95 +4 °C until sent to VEBIO. Rapid and safe shipping practices were used to avoid contamination and

96 ensure samples reached VEBIO within 48 h. At the VEBIO facility, an aliquot was taken from the
97 sample to perform the requested biomedical analyses. Another aliquot was then stored at +4 °C until
98 sent to the MIVEGEC lab, Montpellier, where serological analyses were performed. Safe shipping
99 practices with an approved professional carrier were also used for shipment to the MIVEGEC lab.
100 Finally, the samples were stored at the MIVEGEC lab at −20 °C until testing (Figure 1). For shipping to
101 the CIRI lab, SARS-CoV-2-positive samples detected by MIA were transported by an approved
102 professional carrier at -20°C to ensure optimal safety conditions. Data (age, sex, clinical history, and
103 region localization, when available) from dogs and cats were provided anonymized by VEBIO to the
104 MIVEGEC lab.



105

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

109 **Ethics**

110 According to the act governing the "use of live animals for scientific purposes" effective in France on
111 14 January 2022, ethical approval was not sought or required since all pets were sampled by a
112 veterinarian during a health care visit. All applicable international and national guidelines for the care
113 of pets were followed.

114 **Microsphere Immunoassay (MIA)**

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

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

136 **Neutralization activity measurement**

137 An MLV-based pseudoparticle carrying a GFP reporter pseudotyped with SARS-CoV-2 spike protein
138 (Wuhan-Hu-1 strain) (SARS-CoV-2pp) was used to measure neutralizing antibody activity in cat and
139 dog sera. Each SARS-CoV-2-positive sample detected by MIA was processed according to a
140 neutralization procedure as previously described (Legros et al., 2021). Briefly, for neutralization
141 assays, a sample of ~1 × 10^3 pseudoparticles was incubated with a 100-fold dilution of sera or
142 control antibodies for 1 h at 37 °C before infection of Vero-E6R cells. At 72 h post-transduction, the
143 percentage of GFP-positive cells was determined by flow cytometry (at least 10 000 events
144 recorded). The level of infectivity is expressed as the percentage of GFP-positive cells and compared
145 to cells infected with SARS-CoV-2pp incubated without serum. As a control, the same procedure was
146 done using RD114 pseudoparticles to identify sera with aspecific neutralization. Sera exhibiting more
147 than 30% SARS-CoV-2pp neutralization were considered positive. Pre-pandemic serum from France
148 was used as a negative control, and an anti-SARS-CoV-2 RBD antibody was used as a positive control.

149 **Statistical analyses**

150 Associations between SARS-CoV2 infection status (positive or negative) and the covariates region,
151 age, and sex were assessed using binomial (logistic) generalized linear models. The region was
152 defined by where the animal lived at the time of sampling. Age was that recorded by the
153 veterinarian, with variable precision, generally in months for young animals and whole years for
154 older animals. Its accuracy is unknown. The associated statistical tests were likelihood ratio tests. All
155 analyses were performed using R software (R Development Core Team, 2018).

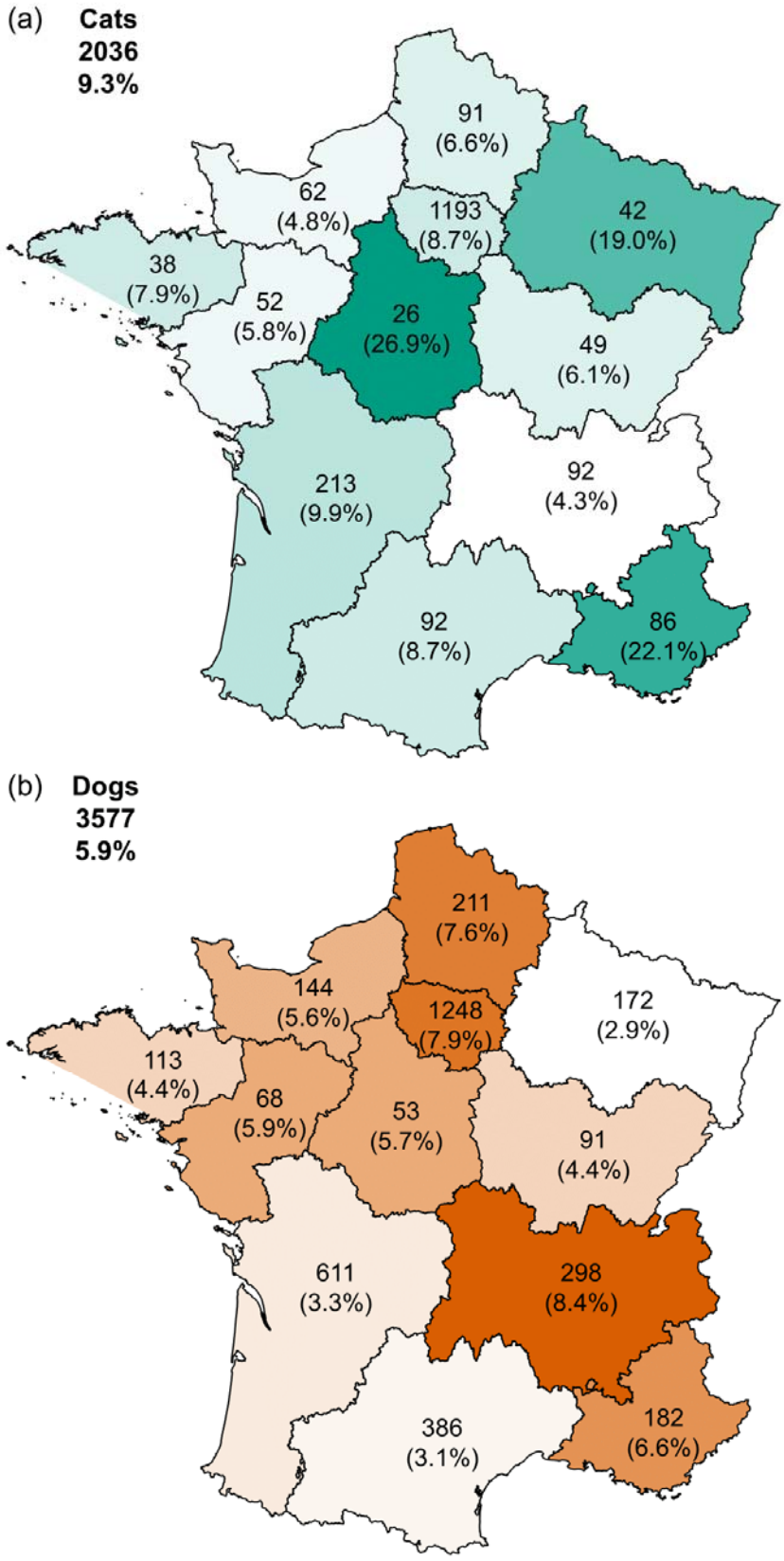
156 **Results**

Blood collection

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

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

	10/2020	11/2020	12/2020	01/2021	02/2021	03/2021	04/2021	05/2021	06/2021
cats	49	256	275	291	225	296	305	166	173
dogs	84	428	543	475	403	474	597	282	291
all	133	684	818	766	628	770	902	448	464


(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%)

171

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

180 **Global seroprevalence**

181 For the sera samples, 401 (7.1%) showed a positive result either against RBD, tri-S, or both
182 (Supplementary Table 1). We next determined the presence of antibodies with neutralizing activity
183 among the positive sera. To save time, we randomly tested approximately 75% (308) of positive sera
184 samples using a retrovirus-based pseudoparticle assay detecting SARS-CoV-2 neutralizing antibodies
185 (Materials and Methods). Pets were considered positive if they showed at least 30% neutralization.
186 Seroneutralizing activity was detected in 81 (26.3%) of the 308 pet sera samples. Among these
187 positive samples, 39 (48%) were positive for both RBD and tri-S, 39 (48%) were positive only for tri-s
188 and 3 (4%) were only positive for RBD. Previous studies have shown that some pets do not develop
189 neutralizing antibodies (Ferasin et al., 2021; Krafft et al., 2021); therefore, only the seroprevalence
190 from MIA assays was analyzed in the remainder of the study.

191 **Seroprevalence in cats and dogs**

192 We observed that a significantly greater proportion of cats were positive (189/2036, 9.3%) than dogs
193 (212/3577, 5.9%); OR = 1.62, 95% c.i. [1.32 - 1.99], P-value = 3.8e-06, Table 1. In addition, sera from
194 MIA-positive cats were more likely to show neutralizing activity (49/144, 34%) than dogs (32/164,
195 19.5%); OR = 2.12, 95% c.i. [1.27 - 3.57], P-value = 0.0039). Species differences were not always

196 significant within each region, likely due to reduced statistical power. However, when differences
197 were significant, it was always the case that cats were more likely to be positive than dogs. (Table 2).

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

204

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 nine 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).

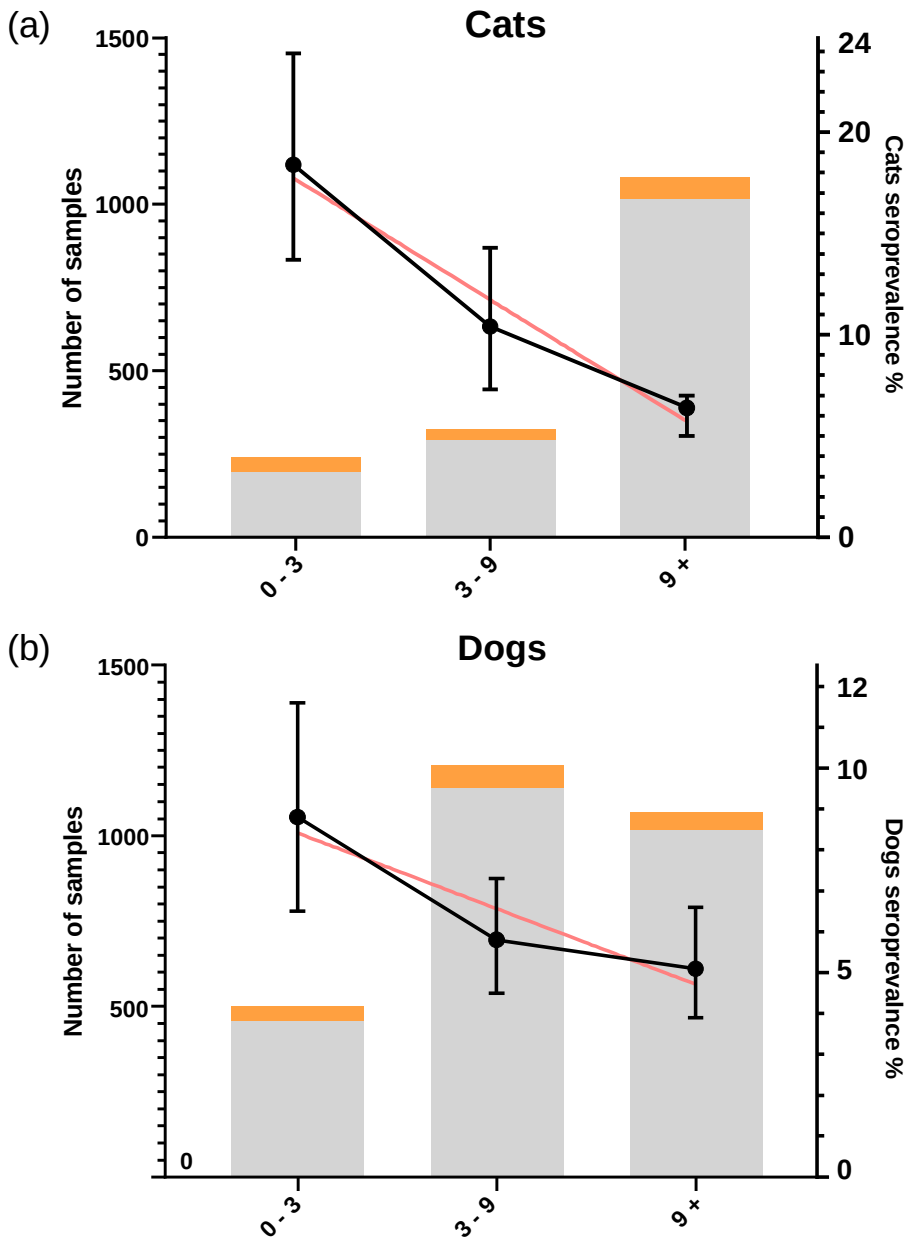


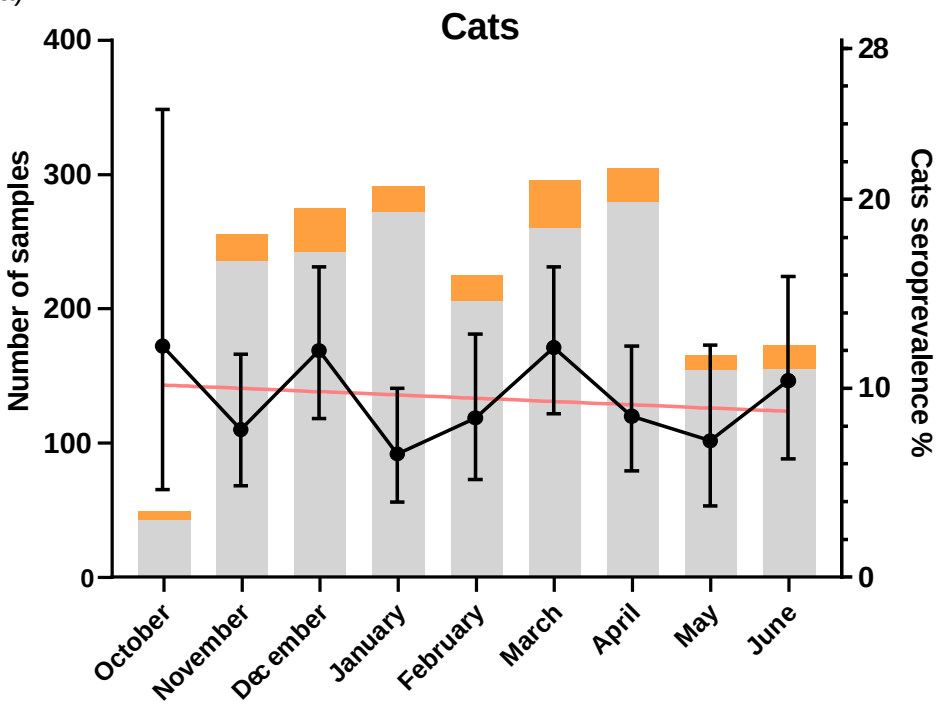
Figure 3. (a). The number of cat blood samples tested by age group for anti-SARS-CoV-2 antibodies by MIA from October 2020 through June 2021. Samples testing negative are shaded grey, and seropositive samples are in orange. Seroprevalence is represented by black dots, with 95 % binomial confidence interval.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

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 intervals. The red line represents the linear regression.

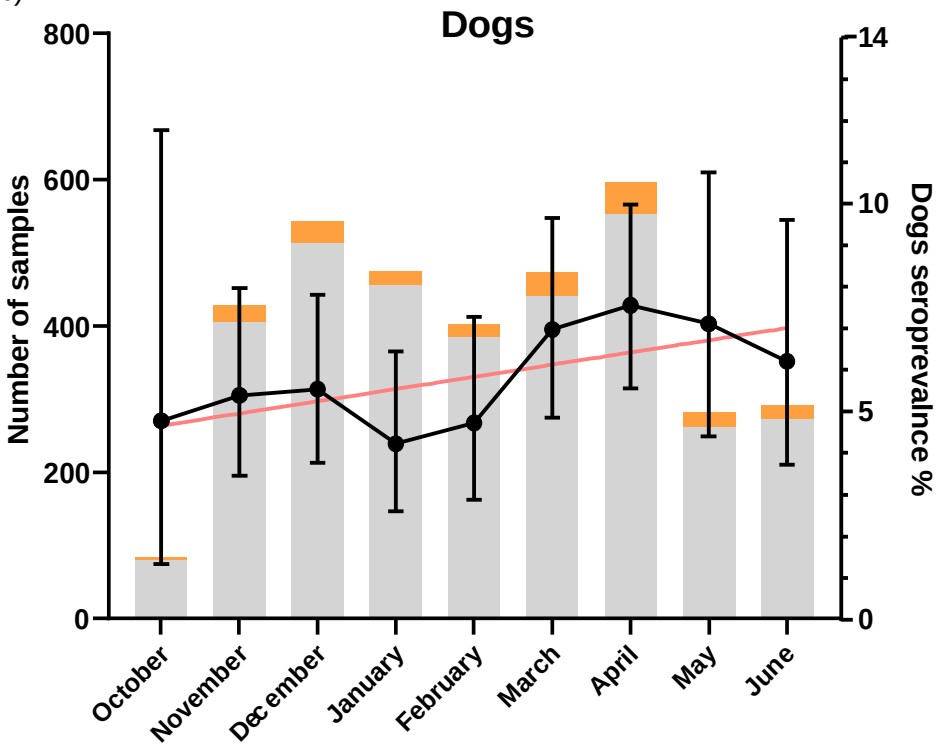
Seroprevalence over time

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

(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 report 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. 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 (Garcia-Beltran et al., 2021).

270 In cats, we found a higher seroprevalence (9.3%) than previously observed in other European
271 countries, which ranged from 0% to 6.4% (Michelitsch et al., 2020; Stevanovic et al., 2020;
272 Barroso-Arévalo et al., 2021; Michelitsch et al., 2021; Pomorska-Mól et al., 2021; Schulz et al., 2021;
273 Smith et al., 2021; Zhao et al., 2021). However, most of these studies were done before the second
274 wave, during a period of relatively lower viral circulation than our sampling period. In addition, most
275 of these studies used a seroneutralisation assay.

276 In dogs, the observed seroprevalence (5.9%) is in accord with a previous study in France showing a
277 prevalence of 4.8% in companion and military working dogs sampled between February 2020 and
278 February 2021 (Laidoudi et al., 2021). Other studies looking for SARS-CoV-2 antibodies in dogs have
279 reported seroprevalences ranging from 0% to 14.5% (Stevanovic et al., 2020; Barroso-Arévalo et al.,
280 2021; Pomorska-Mól et al., 2021; Smith et al., 2021; Stevanovic et al., 2021; Zhao et al., 2021).

281 Importantly, we observed a significantly higher seroprevalence of anti-SARS-CoV-2 antibodies in cats
282 than in dogs (p = 4.2e-08). The statistical significance of this difference varied among regions, likely
283 due to the reduced power and perhaps some unintended sampling bias by veterinarians. For
284 example, the smallest sample size was in the Bourgogne-Franche-Comté region, where we observed
285 no significant difference between dogs and cats. Furthermore, for a region like Ile-de-France, where
286 people live mostly in apartments, we can also hypothesize that dogs live in closer contact with
287 owners than in the rest of France. Previous studies with fewer samples have either found no
288 significant difference between species (Patterson et al., 2020; Stevanovic et al., 2020;
289 Barroso-Arévalo et al., 2021; Calvet et al., 2021; Fritz et al., 2021; Pomorska-Mól et al., 2021) or that
290 cats have significantly higher seroprevalence than dogs (Colitti et al., 2021; Hamer et al., 2021;
291 Barroso et al., 2022).

292 Our study of a very large population of dogs and cats in natural conditions provides some evidence
293 that cats are more susceptible to SARS-CoV-2 infection than dogs, at least during the time frame of
294 our sampling period. Potential causes of species differences in susceptibility between cats and dogs

295 are numerous, but likely include a variety of biological and behavioural factors, as well as differences
296 in exposure. Intererestingly, ACE-2 shows greater sequence similarity between cat and human
297 orthologs than observed between dogs and humans (Damas et al., 2020). The absence of data such
298 as the pet lifestyle (Indoor/Outdoor), or the frequency and nature of contacts with humans and other
299 animals restricts our ability to identify a potential cause of the observed difference. In previously
300 studies, most infected pets were epidemiologically linked to humans who had tested positive for
301 COVID-19 (Maurin et al., 2021).

302 We did not observe significant sex differences in seroprevalence in either species (p = 0.45). Our
303 findings are consistent with most previous studies also reporting an absence of sex differences in
304 dogs and cats (Calvet et al., 2021; Pomorska-Mól et al., 2021; Barroso et al., 2022; Jairak et al., 2022).
305 A smaller study of 188 dogs and 61 cats found higher seropositivity in male dogs and an absence of a
306 sex difference in cats (Patterson et al., 2020). Another study found that male dogs sampled from the
307 general population were more likely to test positive than females, but this difference was not
308 observed in dogs from COVID-19+ households (Stevanovic et al., 2021). There is little evidence of a
309 significant sex difference in susceptibility in humans. However, men are more likely to be affected by
310 severe forms of COVID than women for a variety of reasons (Bechmann et al., 2022).

311 In terms of age, we observed a higher seroprevalence among younger animals (between 0-3 years)
312 for both species that then decreased with age. A study of dogs sampled from the general population
313 found seroprevalence was highest in animals aged 5-6 years and that in COVID-19+ households,
314 seroprevalence peaked in slightly younger dogs, aged between one and five years (Stevanovic et al.,
315 2021). Other studies have reported no significant associations with age in cats and dogs (Pomorska-
316 Mól et al., 2021; Barroso et al., 2022). An experimental study in cats found that juveniles appear
317 more vulnerable than subadults (Shi et al., 2020). The decreasing seroprevalence we observed with
318 age could also arise from age-dependent behavioural changes. For example, young animals (< 3 years
319 old) are more active and curious and may be in greater contact with their owners than older animals

320 that prefer to remain quieter. The decrease could also reflect immunosenescence in older animals, as
321 observed in humans.

322 Interestingly, we observed a slight increase in seroprevalence in dogs during the study's nine months
323 of sampling, a trend not observed among cats. We expected an increase because antibodies have a
324 longer persistence in the organism than viral RNA; thus, animals sampled at later dates would
325 represent an accumulation of cases. The absence of a positive association between seroprevalence
326 and the time of sampling in cats has been reported in two other studies in Europe, but conclusions
327 were limited by the small number of samples collected over just a few months (Adler et al.; Schulz et
328 al., 2021). The absence of an association in cats suggests a limited persistence of antibodies in cats
329 than dogs. Few studies have investigated variation in the persistence of antibodies in animals. For
330 example, a study carried out on seven dogs and two cats infected in natural conditions showed
331 persistence of neutralizing antibodies up to 10 months after infection in four of the dogs and the two
332 cats, but also that persistence was markedly reduced in two of the dogs after three months (Decaro
333 et al., 2021). Moreover, a study of two cats found that neutralizing antibodies had disappeared by
334 110 days (Zhang et al., 2020). Based on these data, one possible reason for the lack of increase in
335 seroprevalence during our study period could be a progressive seroreversion of infected cats that is
336 equally compensated by the number of new infections, i.e. seroconversion. If so, this would mean
337 that the observed seroprevalence is not an accurate reflection of the total number of infections, at
338 least in cats, during the whole epidemic. Instead, seroprevalence provides a snapshot if infections
339 acquired during a time period. This also suggests that the seroprevalence observed in our study may
340 underestimate the actual proportion of cats infected during the entirety of the epidemic. In order to
341 enhance our comprehension of antibody persistence in cats and dogs, a longitudinal serological study
342 involving a larger number of cats and dogs has to be conducted.

343 Human-to-pet transmission may promote viral adaptation facilitating re-infection with novel viral
344 strains in humans (Bashor et al., 2021). While one case of infection from cat to human has recently

been reported, the large number of pet cats and their frequent close interaction with humans provides ample opportunity. This possibility raises the question of a vaccination strategy for animals susceptible to SARS-CoV-2 infection. While pets do not currently seem to play a role in the ongoing pandemic, our results emphasize the magnitude of SARS-CoV-2 infection in pets is not trivial. Combined with the size of domestic cat and dog populations and the close contact with their human companions, our results highlight the importance of collecting more data on SARS-CoV-2 transmissibility and pathogenicity in companion animals, especially with the emergence of new variants. Also, when a SARS-CoV-2 infection is suspected in a pet, we suggest collecting a sample for RT-qPCR confirmation 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.

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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.

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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.

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