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 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 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 showing that cats are more susceptible than dogs. This study reinforces that pets are commonly infected or exposed to SARS-CoV-2, emphasizing the importance of a One-Health approach to the SARS-CoV-2 pandemic and raising the question of vaccination of companion animals in close contact with humans.

Introduction

Two months after the onset of SARS-CoV-2 circulation in the human population, the first cases of natural SARS-CoV-2 infection in pets were officially reported in Hong Kong in two dogs (Sit et al., 2020). Since then, viral RNA and SARS-CoV-2 antibodies have been detected in several dogs and cats, mostly

belonging to COVID-19 infected owners (Barrs et al., 2020; Hamer et al., 2021; Jairak et al., 2021; Krafft et al., 2021). Furthermore, several studies have demonstrated that the risk of pets testing seropositive was higher in COVID-19+ households than for pets from households of unknown status (Barroso, Vieira-Pires, Antunes, & Fidalgo-Carvalho, 2022; Colitti et al., 2021; Fritz et al., 2021; Patterson et al., 2020; Stevanovic et al., 2021).

A recent study from Thailand reports a suspected case of SARS-CoV-2 transmission from a cat to a human (Sila et al., 2022). Dog-to-human transmission has yet to be described. Given that 200 million cats and dogs live in close proximity to humans in Europe (The European Pet Food Industry, 2020), there is ample opportunity for such transmission, from cats or dogs, and the potential risks need to be carefully considered.

Several population-based serological studies of dogs and cats have found SARS-CoV-2 antibodies. In dogs, seroprevalence has ranged from 0% to 14.5% (Barroso-Arévalo et al., 2021; Barroso et al., 2022; Barua et al., 2021; Dileepan et al., 2021; Laidoudi et al., 2021; Pomorska-Mól et al., 2021; Smith et al., 2021; Stevanovic et al., 2021; Stevanovic et al., 2020; Udom et al., 2021; Zhao et al., 2021). In cats, seroprevalence has ranged from 0% to 21.7% (Barroso et al., 2022; Barua et al., 2021; Dileepan et al., 2021; A. Michelitsch, Hoffmann, Wernike, & Beer, 2020; Anna Michelitsch, Schön, Hoffmann, Beer, & Wernike, 2021; Schulz et al., 2021; Udom et al., 2021; Zhang et al., 2020). Among these studies, five directly compared cat and dog seroprevalence. Some experimental and epidemiological studies suggest that cats are more susceptible to infection than dogs (Barroso et al., 2022; Bosco-Lauth et al., 2020; Colitti et al., 2021; Dileepan et al., 2021; Shi et al., 2020), although significant species differences have not always been observed (Barroso-Arévalo et al., 2021; Pomorska-Mól et al., 2021; Stevanovic et al., 2020). For both species, seroprevalence was highly dependent on the period of sampling (first, second wave *etc.*), the assay used (ELISA, seroneutralization, etc.), and the country of sampling (China, Croatia, Germany, Italy, Netherland, Poland, Portugal, Spain, Thailand, United-Kingdom, USA). Moreover, most of these studies had important limitations (low number of enrolled animals, a short

72 sampling period, etc.), curtailing their ability to reach definitive conclusions on the frequency and
73 impact of pet infections on COVID-19 epidemiology.

74 To provide more data about SARS-CoV-2 infection in companion animals, we have conducted a large-
75 scale serological survey of SARS-CoV-2 infections in cats and dogs from October 2020 through June
76 2021 living in French households.

77 **Materials and Methods**

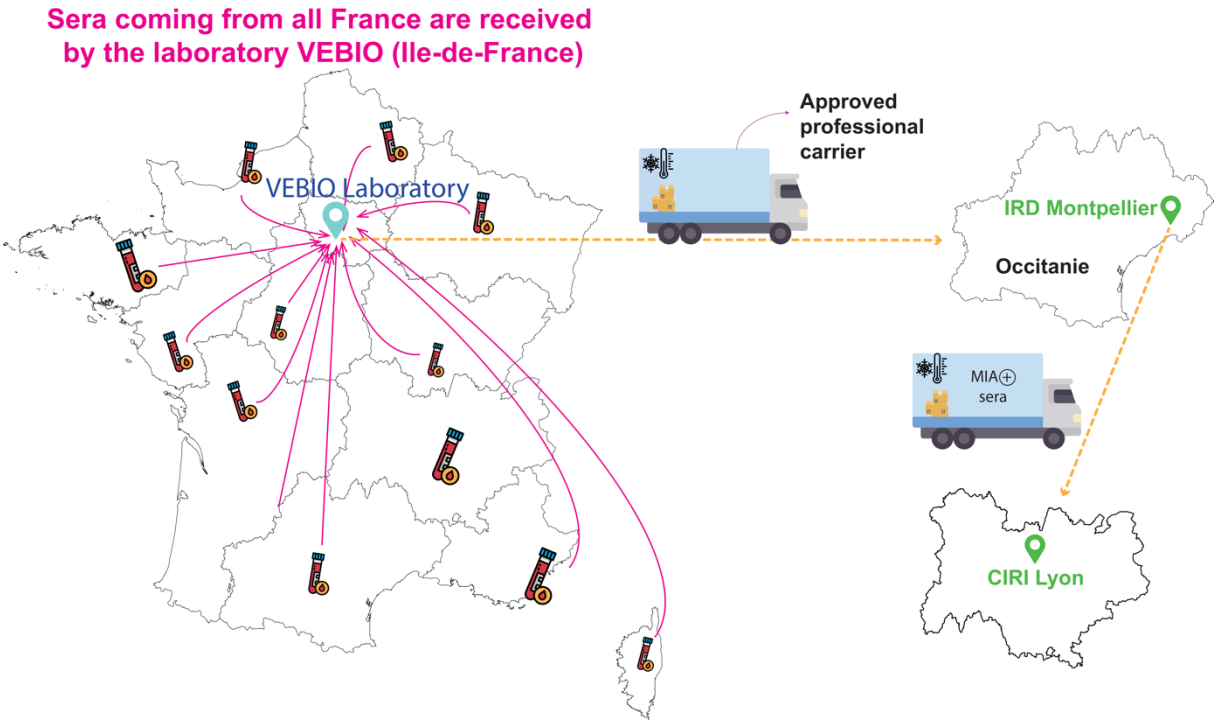
78 **Sampling**

79 Nation-wide sampling was possible thanks to a network of veterinary clinics spread across France
80 working with VEBIO, a veterinary diagnostic laboratory. No inclusion and exclusion criteria were
81 applied for the selection of blood samples, except that they came only from veterinary clinics working
82 with VEBIO. VEBIO notified the veterinary clinics that serum remaining following requested biomedical
83 analyses could be used in a SARS-CoV-2 research project. No specific request for samples was
84 addressed to the vets. Thus, the SARS-CoV-2 analysis is based on samples collected during the regular
85 activities of the vets.

86 In detail, sample collection was done as follows. Blood samples were collected in dry/EDTA tubes from
87 dogs and cats during routine healthcare visits or for diagnostic purposes at veterinary clinics. After
88 centrifugation, the serum/plasma was kept at +4 °C until it was sent to VEBIO. Shipping was conducted
89 under safe and rapid conditions to avoid contamination and ensure samples reached VEBIO within 48
90 h. Next, an aliquot was taken from the sample to perform the requested biomedical analyses. Another
91 aliquot was stored at +4 °C until being sent to the MIVEGEC lab, Montpellier, where serological
92 analyses were carried out. For shipping to the MIVEGEC lab, all samples were placed at +4 °C and
93 transported by an approved professional carrier to ensure optimal safety conditions. Finally, the
94 samples were stored at the MIVEGEC lab at −20 °C until testing (Figure 1). For shipping to the CIRI lab,
95 SARS-CoV-2 positive samples detected by MIA were transported by an approved professional carrier

96 at -20°C to ensure optimal safety conditions. Data (age, sex, clinical history, region localization when

97 available) from dogs and cats were provided anonymized by VEBIO to the MIVEGEC lab.



98

99 **Figure 1. Logistics of sample collection and distribution in the study.** Sera collected during routine

100 healthcare visits by veterinarians throughout France were first sent to VEBIO in Ile-de-France. Aliquots

101 of the samples were then made and sent to the IRD in Montpellier (Hérault) via an approved

102 transporter.

103 **Ethics**

104 According to the act governing the "use of live animals for scientific purposes" effective in France on

105 14 January 2022, ethical approval was not sought or required since all pets were sampled by a

106 veterinarian during a health care visit. All applicable international and national guidelines for the care

107 of pets were followed.

108 **Microsphere Immunoassay (MIA)**

109 Dog and cat serum samples were tested using a multiplex microsphere immunoassay (MIA). Ten µg of

110 two recombinant SARS-CoV-2 antigens, receptor-binding domain (RBD) and trimeric spike (tri-S) both

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

126 **Neutralization activity measurement**

127 An MLV-based pseudoparticle carrying a GFP reporter pseudotyped with SARS-CoV-2 spike protein
128 (Wuhan-Hu-1 strain) (SARS-CoV-2pp) was used to measure the neutralizing antibody activity in cat and
129 dog sera. Each SARS-CoV-2 positive sample detected by MIA was processed according to a
130 neutralization procedure as previously described (Legros et al., 2021). Briefly, for neutralization assays,
131 a sample of ~1×10^3 pseudoparticles was incubated with a 100-fold dilution of sera or control
132 antibodies for 1 h at 37 °C before infection of Vero-E6R cells. At 72 h post-transduction, the percentage
133 of GFP-positive cells was determined by flow cytometry (at least 10 000 events recorded). The level of
134 infectivity is expressed as the percentage of GFP-positive cells and compared to cells infected with
135 SARS-CoV-2pp incubated without serum. As a control, the same procedure was done using RD114

136 pseudoparticles to identify sera with unspecific neutralization. Sera exhibiting more than 30% SARS-
137 CoV-2pp neutralization were considered positive. Pre-pandemic serum from France was used as a
138 negative control, and an anti-SARS-CoV-2 RBD antibody was used as a positive control.

139 **Statistical analyses**

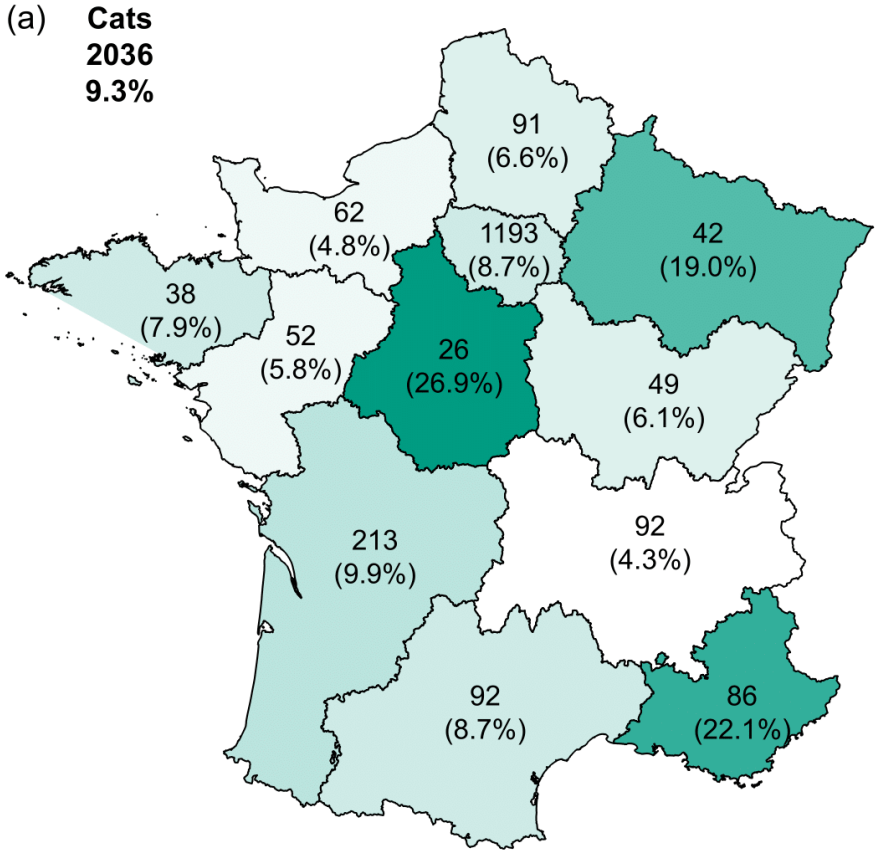
140 Fisher's exact test and binomial generalized linear models have been used. All computations were
141 performed with the R software (R Development Core Team, 2018).

142 **Results**

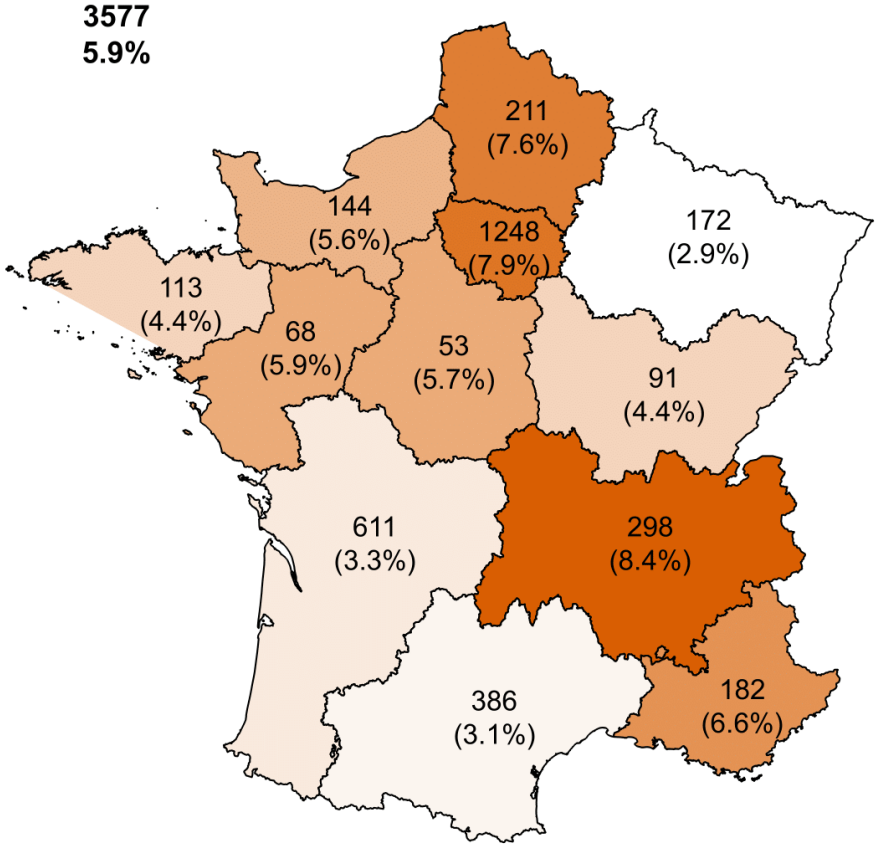
143 **Blood collection**

144 Blood samples of 2036 cats and 3577 dogs were collected during routine healthcare visits by
145 veterinarians from October 2020 through June 2021. Samples came from all 13 regions of metropolitan
146 France. Corsica was then excluded due to too few samples. Almost half of the samples came from Ile-
147 de-France, the region including Paris, reflecting population density and proximity to the Veterinary
148 diagnostic laboratory (VEBIO), where all samples for biomedical analyses requested by the
149 veterinarians were handled (Materials and Methods). The number of remaining samples received from
150 the other French regions largely depended on the number of veterinarians working with VEBIO in those
151 regions (Figure 2). When available, each animal's age, sex, and location were provided anonymized by
152 VEBIO. Unfortunately, we could not study the clinical history of the animals due to variability in how
153 each veterinarian reported this information.

(a) **Cats**
2036
9.3%



(b) **Dogs**
3577
5.9%



155

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

164 **Global seroprevalence**

165 All 5613 sera samples were tested by Microsphere Immunoassay (MIA) against Receptor-binding
166 domain (RBD) and trimeric Spike (tri-S) antigens. Specific seropositivity cut-offs were established for
167 each antigen and species (Materials and Methods). To account for isotypic variability and the excellent
168 specificity observed for both antigens, an animal was determined to be positive for SARS-CoV-2
169 antibodies following a positive result for at least one of the two tests.

170 For the sera samples, 401 (7.1%) showed a positive result either against RBD, tri-S, or both
171 (Supplementary Table 1). We next determined the presence of antibodies with neutralizing activity
172 among the positive sera. To save time, we randomly tested approximately 75% (308) of positive sera
173 samples using a retrovirus-based pseudoparticle assay detecting SARS-CoV-2 neutralizing antibodies
174 (Materials and Methods). Pets were considered positive if they showed at least 30% of neutralization.
175 Seroneutralizing activity was detected in 81 (26.3%) of the 308 pet sera samples. Among them, 39
176 (48%) are positive for both RBD and tri-S, 39 (48%) are positive only for tri-s and 3 (04%) are only
177 positive for RBD. Previous studies have shown that some pets did not develop neutralizing antibodies
178 (Ferasin et al., 2021; Krafft et al., 2021) therefore, only the seroprevalence from MIA assays was
179 analyzed in the remainder of the study.

180 **Seroprevalence in cats and dogs**

181 We observed a significantly greater proportion of cats were positive (189/2036, 9.3%) than dogs
182 (212/3577, 5.9%; Fisher's test: *p* = 4.2e-08, Table 1). In addition, sera from MIA-positive animals were
183 more likely to show neutralizing activity in cats (49/144, 34%) than in dogs (32/164, 19.5%; Fisher's
184 test: p = 0.0044). Species differences were not always significant within each region, likely due to
185 reduced power. However, when differences were significant, it was always the case that cats were
186 more likely to be positive than dogs. (Table 1).

187 **Table 1.** Seroprevalence of IgG SARS-CoV-2 antibodies in blood samples from cats and dogs by French
188 region from October 2020 through June 2021.

Region	Cats	Cats seroprevalence		Dogs	Dogs seroprevalence		P-value
Auvergne-Rhône-Alpes	4/92	4.3%	(1.2-10.8)	25/298	8.4%	(5.5-12.1)	0.26
Bourgogne-Franche-Comté	3/49	6.1%	(1.3-16.9)	4/91	4.4%	(1.2-10.9)	0.70
Bretagne	3/38	7.9%	(1.7-21.4)	5/113	4.4%	(1.5-10.0)	0.42
Centre-Val de Loire	7/26	26.9%	(11.6-47.8)	3/53	5.7%	(1.2-15.7)	0.012
Grand Est	8/42	19.0%	(8.6-34.1)	5/172	2.9%	(1.0-6.7)	0.00072
Hauts-de-France	6/91	6.6%	(2.5-13.8)	16/211	7.6%	(4.4-12.0)	1.0
Île-de-France	104/1193	8.7%	(7.2-10.5)	98/1248	7.9%	(6.4-9.5)	0.46
Normandie	3/62	4.8%	(1.0-13.5)	8/144	5.6%	(2.4-10.7)	1.0
Nouvelle-Aquitaine	21/213	9.9%	(6.2-14.7)	20/611	3.3%	(2.0-5.0)	0.00037
Occitanie	8/92	8.7%	(3.8-16.4)	12/386	3.1%	(1.6-5.4)	0.036
Provence-Alpes-Côte d'Azur	19/86	22.1%	(13.9-32.3)	12/182	6.6%	(3.5-11.2)	0.00040

Pays de la Loire	3/52	5.8%	(1.2-15.9)	4/68	5.9%	(1.6-14.4)	1.0
Total	**189/2036**	**9.3%**	**(8.0 ; 10.6)**	**212/3577**	**5.9%**	**(5.2 ; 6.8)**	**$4.2.10^{-6}$**

Data are presented as No. positive, percentage (95 % exact binomial confidence intervals). P-values correspond to the Fisher exact test comparing seroprevalence in cats vs dogs.

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 = 0.45) or among cats (females 9.4%; 78/827, males 9.8%; 99/1009, p = 0.81) and dogs (females: 5.5%; 85/1534, males: 6.2%; 113/1833) tested separately (Table 2).

Table 2. Seroprevalence of IgG SARS-CoV-2 antibodies in blood samples from cats and dogs by sex from October 2020 through June 2021.

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)**
P-value		0.81		0.46		0.45

Data are presented as No. positive, percentage (95 % exact binomial confidence intervals). P-values correspond to the Fisher exact test comparing seroprevalence in cats vs dogs.

Seroprevalence by age

The age of 1657 cats (age range 0.2 – 22yr) and 2781 dogs (age range 0.1 – 18.5yr) was known. Among cats, 18.4% aged 0-3 years, 10.4% aged 4-9 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 (p-value = 3.9e-08) and dogs (p-value = 0.016) (Figure 3) (Supplementary Table 2).

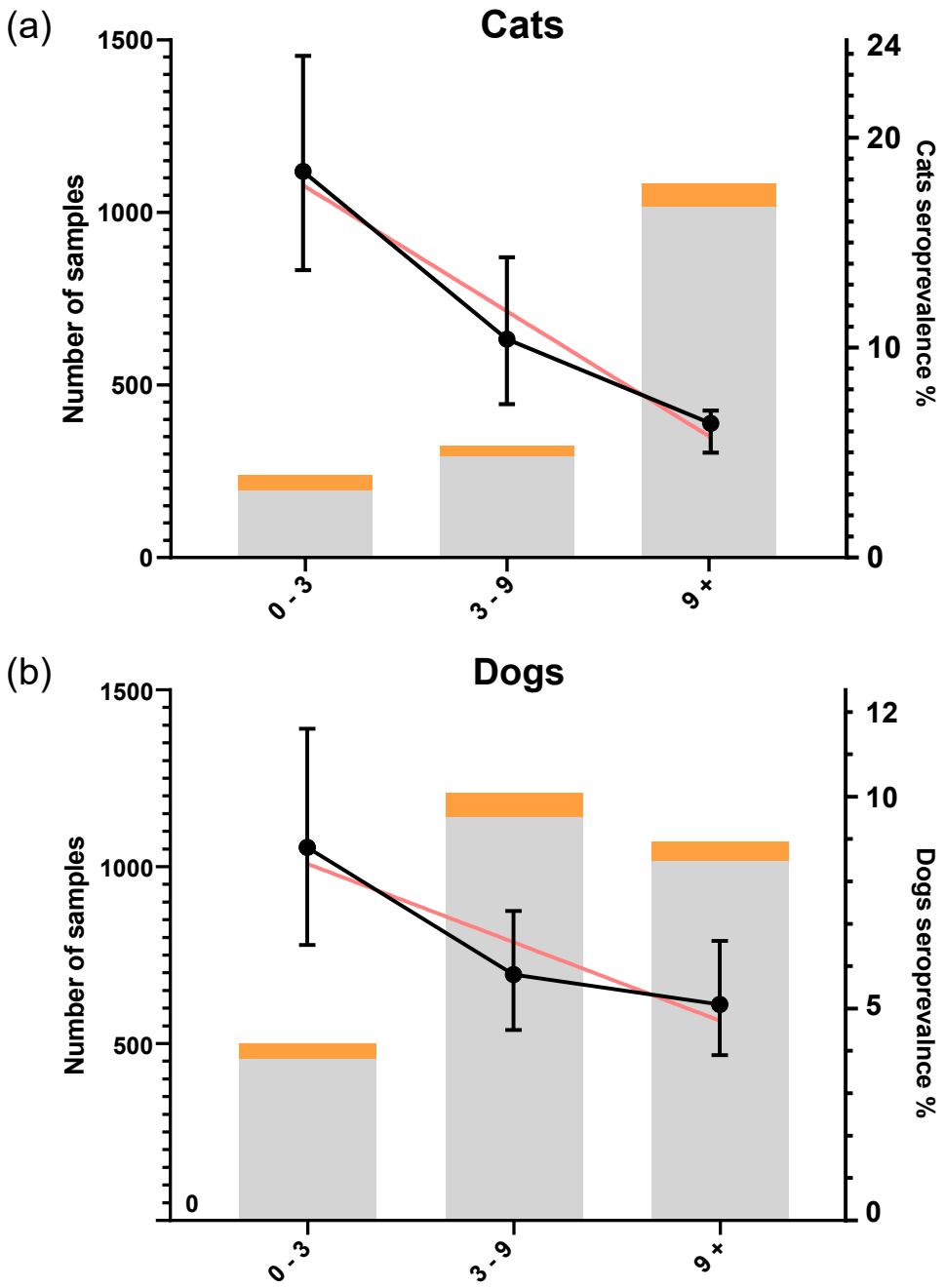
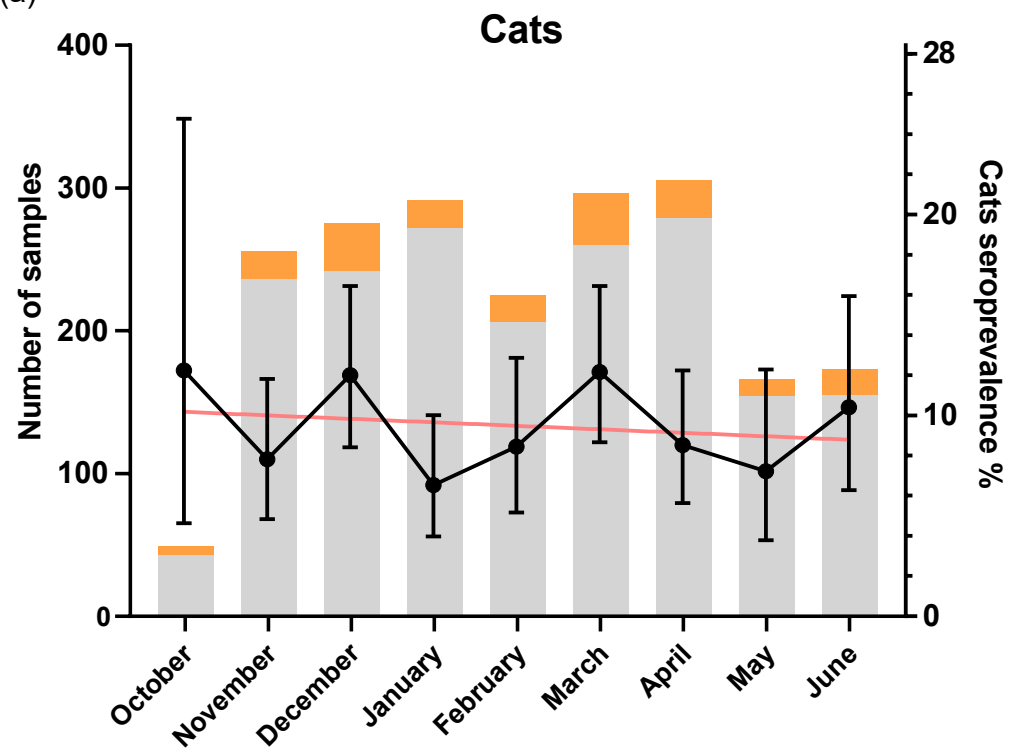


Figure 3. (a). The number of cat blood samples tested by age group for anti-SARS-CoV-2 antibodies by MIA from October 2020 through June 2021. Samples testing negative are shaded grey, and seropositive samples are in orange. Seroprevalence is represented by black dots, with 95 % binomial confidence interval.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

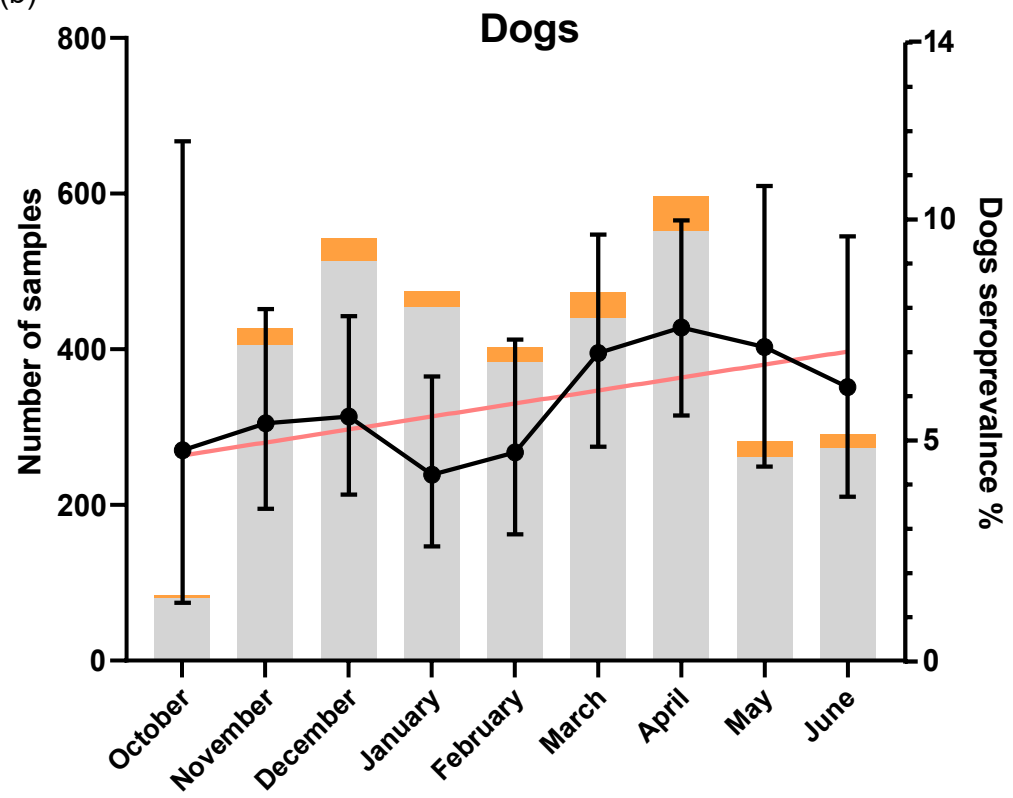
Seroprevalence over time

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 (linear trend, cats : p-value = 0.88, dogs : p-value = 0.057).

(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.

Discussion

This study reports a large-scale serological survey of pet (cats and dogs) sera 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 nearly 6000 pets, we report a seroprevalence of anti-SARS-CoV-2 antibodies of 7.1%. We observed that only a small percentage of samples (28%) is positive for both tri-s and RBD, indicating that our RBD assay seems to be less sensitive than the tri-s assay. This may be explained by the fact that the full trimeric spike antigen might detect a broader range of antibodies than the receptor-binding domain which represent only a small part of the spike protein. Furthermore, we demonstrated that only 26% of seropositive pets demonstrated neutralizing antibody activity. Moreover, we have shown that cats are more likely to produce neutralizing antibodies. This is likely associated with a more prolonged and intense immune stimulation than dogs. In humans, a study has shown that disease severity is positively correlated with neutralizing antibody levels (Garcia-Beltran et al., 2021).

In cats, we found a higher seroprevalence (9.3%) than previously observed in other European countries, which ranged from 0% to 6.4% (Barroso-Arévalo et al., 2021; A. Michelitsch et al., 2020;

248 Anna Michelitsch et al., 2021; Pomorska-Mól et al., 2021; Schulz et al., 2021; Smith et al., 2021;
249 Stevanovic et al., 2020; Zhao et al., 2021). However, most of these studies were done before the
250 second wave, a period of relatively lower viral circulation than our sampling period. In addition, most
251 of these studies used a seroneutralisation assay.

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

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

267 Our study of a very large population of dogs and cats in natural conditions provides some evidence
268 that cats are more susceptible to SARS-CoV-2 infection than dogs, at least during the time frame of our
269 sampling period. Potential causes of species differences in susceptibility between cats and dogs are
270 numerous, likely including biological (ACE-2 shows greater similarity between cat and human than
271 between dogs and humans orthologs (Damas et al., 2020)) and behavioural parameters. The absence
272 of data such as the neuter status, pet lifestyle (Indoor/Outdoor), or the frequency and nature of

contacts with humans and other animals restricts our ability to identify a potential cause of the observed difference. In previously published cases, most infected pets were epidemiologically linked to humans who tested positive for COVID-19 (Maurin et al., 2021).

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

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

298 Interestingly, we did not observe a significant increase in seroprevalence over the eight months of our
299 study. We expected such an increase because antibodies have a longer persistence in the organism
300 than viral RNA; thus, animals sampled at later dates would represent an accumulation of cases. This
301 finding has also been observed in two other studies in Europe, but conclusions were limited by the
302 small number of samples collected over just a few months (Adler et al.; Schulz et al., 2021). The absence
303 of a positive association between seroprevalence and the time of sampling could be explained by the
304 limited persistence of antibodies in pets. Few studies have investigated the persistence of antibodies
305 in animals. For example, a study carried out on seven dogs and two cats infected in natural conditions
306 showed persistence of neutralizing antibodies up to 10 months after infection in four of the dogs and
307 the two cats, but also that persistence was markedly reduced in two of the dogs after three months
308 (Decaro et al., 2021). Moreover, a study of two cats found that neutralizing antibodies had disappeared
309 by 110 days (Zhang et al., 2020). Based on these data, one possible reason for the lack of increase in
310 seroprevalence during our study period could be a progressive seroreversion of infected animals that
311 is equally compensated by the number of new infections, i.e. seroconversion. If so, this would mean
312 that the observed seroprevalence is not an accurate reflection of the total number of infections in cats
313 and dogs during the whole epidemic but rather a snapshot of a time period that remains to be defined
314 by longitudinal serological studies involving a larger number of animals. This also suggests that the
315 seroprevalence observed in our study may underestimate the actual proportion of animals infected
316 during the entirety of the epidemic.

317 Human-to-pet transmission may promote viral adaptation facilitating re-infection with novel viral
318 strains in humans (Bashor et al., 2021). While one case of infection from cat to human has recently
319 been reported, the large number of pet cats and their frequent close interaction with humans provides
320 ample opportunity. This possibility raises the question of a vaccination strategy for animals susceptible
321 to SARS-CoV-2 infection. While pets do not currently seem to play a role in the ongoing pandemic, our
322 results emphasize the magnitude of SARS-CoV-2 infection in pets is not trivial. Combined with the size
323 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.

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

Adler, J. M., Weber, C., Wernike, K., Michelitsch, A., Friedrich, K., Trimpert, J., . . . Müller, E. 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). doi:https://doi.org/10.1111/zph.12932

Barroso-Arévalo, S., Barneto, A., Ramos, Á. M., Rivera, B., Sánchez, R., Sánchez-Morales, L., . . . Sánchez-Vizcaíno, J. M. (2021). Large-scale study on virological and serological prevalence of SARS-CoV-2 in cats and dogs in Spain. *Transbound Emerg Dis*. doi:10.1111/tbed.14366

Barroso, R., Vieira-Pires, A., Antunes, A., & Fidalgo-Carvalho, I. (2022). Susceptibility of Pets to SARS-CoV-2 Infection: Lessons from a Seroepidemiologic Survey of Cats and Dogs in Portugal. *Microorganisms, 10*(2), 345. Retrieved from https://www.mdpi.com/2076-2607/10/2/345

Barrs, V., Peiris, M., Tam, K. W. S., Law, P. Y. T., Brackman, C., To, E. M. W., . . . Sit, T. H. C. (2020). SARS-CoV-2 in Quarantined Domestic Cats from COVID-19 Households or Close Contacts, Hong Kong, China. *Emerging Infectious Disease journal, 26*(12), 3071. doi:10.3201/eid2612.202786

Barua, S., Hoque, M., Adekanmbi, F., Kelly, P., Jenkins-Moore, M., Torchetti, M. K., . . . Wang, C. (2021). Antibodies to SARS-CoV-2 in dogs and cats, USA. *Emerging Microbes & Infections*, 1-20. doi:10.1080/22221751.2021.1967101

Bashor, L., Gagne, R. B., Bosco-Lauth, A. M., Bowen, R. A., Stenglein, M., & VandeWoude, S. (2021). SARS-CoV-2 evolution in animals suggests mechanisms for rapid variant selection. *Proceedings of the National Academy of Sciences, 118*(44), e2105253118. doi:doi:10.1073/pnas.2105253118

Bechmann, N., Barthel, A., Schedl, A., Herzig, S., Varga, Z., Gebhard, C., . . . Bornstein, S. R. (2022). Sexual dimorphism in COVID-19: potential clinical and public health implications. *The Lancet Diabetes & Endocrinology*. doi:10.1016/s2213-8587(21)00346-6

Bosco-Lauth, A. M., Hartwig, A. E., Porter, S. M., Gordy, P. W., Nehring, M., Byas, A. D., . . . Bowen, R. A. (2020). Experimental infection of domestic dogs and cats with SARS-CoV-2: Pathogenesis, transmission, and response to reexposure in cats. *Proceedings of the National Academy of Sciences, 117*(42), 26382-26388. doi:10.1073/pnas.2013102117

Calvet, G. A., Pereira, S. A., Ogrzewalska, M., Pauvolid-Corrêa, A., Resende, P. C., Tassinari, W. d. S., . . . Menezes, R. C. (2021). Investigation of SARS-CoV-2 infection in dogs and cats of humans

diagnosed with COVID-19 in Rio de Janeiro, Brazil. *PLoS One, 16*(4), e0250853. doi:10.1371/journal.pone.0250853

Colitti, B., Bertolotti, L., Mannelli, A., Ferrara, G., Vercelli, A., Grassi, A., . . . Rosati, S. (2021). Cross-Sectional Serosurvey of Companion Animals Housed with SARS-CoV-2–Infected Owners, Italy. *Emerging Infectious Disease journal, 27*(7). doi:10.3201/eid2707.203314

Damas, J., Hughes Graham, M., Keough Kathleen, C., Painter Corrie, A., Persky Nicole, S., Corbo, M., . . . Lewin Harris, A. (2020). Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates. *Proceedings of the National Academy of Sciences, 117*(36), 22311-22322. doi:10.1073/pnas.2010146117

Decaro, N., Grassi, A., Lorusso, E., Patterson, E. I., Lorusso, A., Desario, C., . . . Buonavoglia, C. (2021). Long-term persistence of neutralizing SARS-CoV-2 antibodies in pets. *Transbound Emerg Dis*. doi:10.1111/tbed.14308

Dileepan, M., Di, D., Huang, Q., Ahmed, S., Heinrich, D., Ly, H., & Liang, Y. (2021). Seroprevalence of SARS-CoV-2 (COVID-19) exposure in pet cats and dogs in Minnesota, USA. *Virulence, 12*(1), 1597-1609. doi:10.1080/21505594.2021.1936433

Ferasin, L., Fritz, M., Ferasin, H., Becquart, P., Corbet, S., Ar Gouilh, M., . . . Leroy, E. M. (2021). Infection with SARS-CoV-2 variant B.1.1.7 detected in a group of dogs and cats with suspected myocarditis. *Vet Rec, 189*(9), e944. doi:https://doi.org/10.1002/vetr.944

Fritz, M., Rosolen, B., Krafft, E., Becquart, P., Elguero, E., Vratskikh, O., . . . Leroy, E. M. (2021). High prevalence of SARS-CoV-2 antibodies in pets from COVID-19+ households. *One health (Amsterdam, Netherlands), 11*, 100192. doi:10.1016/j.onehlt.2020.100192

Garcia-Beltran, W. F., Lam, E. C., Astudillo, M. G., Yang, D., Miller, T. E., Feldman, J., . . . Balazs, A. B. (2021). COVID-19-neutralizing antibodies predict disease severity and survival. *Cell, 184*(2), 476-488.e411. doi:10.1016/j.cell.2020.12.015

Hamer, S. A., Pauvolid-Corrêa, A., Zecca, I. B., Davila, E., Auckland, L. D., Roundy, C. M., . . . Hamer, G. L. (2021). SARS-CoV-2 Infections and Viral Isolations among Serially Tested Cats and Dogs in Households with Infected Owners in Texas, USA. *Viruses, 13*(5), 938. Retrieved from https://www.mdpi.com/1999-4915/13/5/938

Jairak, W., Charoenkul, K., Chamsai, E., Udom, K., Chaiyawong, S., Bunpapong, N., . . . Amonsin, A. (2021). First cases of SARS-CoV-2 infection in dogs and cats in Thailand. *Transbound Emerg Dis*. doi:10.1111/tbed.14383

Jairak, W., Charoenkul, K., Chamsai, E., Udom, K., Chaiyawong, S., Hangsawek, A., . . . Amonsin, A. (2022). 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*. doi:10.1111/zph.12907

Krafft, E., Denolly, S., Boson, B., Angelloz-Pessey, S., Levaltier, S., Nesi, N., . . . Legros, V. (2021). 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, 13*(9), 1759. doi:https://doi.org/10.3390/v13091759

Laidoudi, Y., Sereme, Y., Medkour, H., Watier-Grillot, S., Scandola, P., Ginesta, J., . . . Davoust, B. (2021). SARS-CoV-2 antibodies seroprevalence in dogs from France using ELISA and an automated western blotting assay. *One health (Amsterdam, Netherlands), 13*, 100293. doi:10.1016/j.onehlt.2021.100293

Legros, V., Denolly, S., Vogrig, M., Boson, B., Siret, E., Rigaill, J., . . . Pozzetto, B. (2021). A longitudinal study of SARS-CoV-2-infected patients reveals a high correlation between neutralizing antibodies and COVID-19 severity. *Cellular & Molecular Immunology, 18*(2), 318-327. doi:10.1038/s41423-020-00588-2

Maurin, M., Fenollar, F., Mediannikov, O., Davoust, B., Devaux, C., & Raoult, D. (2021). Current Status of Putative Animal Sources of SARS-CoV-2 Infection in Humans: Wildlife, Domestic Animals and Pets. *Microorganisms, 9*(4). doi:10.3390/microorganisms9040868

Michelitsch, A., Hoffmann, D., Wernike, K., & Beer, M. (2020). Occurrence of Antibodies against SARS-CoV-2 in the Domestic Cat Population of Germany. *Vaccines (Basel), 8*(4). doi:10.3390/vaccines8040772

Michelitsch, A., Schön, J., Hoffmann, D., Beer, M., & Wernike, K. (2021). The Second Wave of SARS-CoV-2 Circulation—Antibody Detection in the Domestic Cat Population in Germany. *Viruses, 13*(6), 1009. Retrieved from https://www.mdpi.com/1999-4915/13/6/1009

Patterson, E. I., Elia, G., Grassi, A., Giordano, A., Desario, C., Medardo, M., . . . Decaro, N. (2020). Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. *Nature Communications, 11*(1), 6231. doi:10.1038/s41467-020-20097-0

Pomorska-Mól, M., Turlewicz-Podbielska, H., Gogulski, M., Ruszkowski, J. J., Kubiak, M., Kuriga, A., . . . Postrzech, M. (2021). A cross-sectional retrospective study of SARS-CoV-2 seroprevalence in domestic cats, dogs and rabbits in Poland. *BMC Veterinary Research, 17*(1), 322. doi:10.1186/s12917-021-03033-2

R Development Core Team. (2018). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Schulz, C., Martina, B., Mirolo, M., Müller, E., Klein, R., Volk, H., . . . Osterhaus, A. (2021). SARS-CoV-2–Specific Antibodies in Domestic Cats during First COVID-19 Wave, Europe. *Emerging Infectious Disease journal, 27*(12). doi:10.3201/eid2712.211252

Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Huang, B., . . . Bu, Z. (2020). Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. *Science, 368*(6494), 1016-1020. doi:10.1126/science.abb7015

Sila, T., Sunghan, J., Laochareonsuk, W., Surasombatpattana, S., Kongkamol, C., Ingviya, T., . . . Chusri, S. (2022). Suspected Cat-to-Human Transmission of SARS-CoV-2, Thailand, July-September 2021. *Emerg Infect Dis, 28*(7), 1485-1488. doi:10.3201/eid2807.212605

Sit, T. H. C., Brackman, C. J., Ip, S. M., Tam, K. W. S., Law, P. Y. T., To, E. M. W., . . . Peiris, M. (2020). Infection of dogs with SARS-CoV-2. *Nature, 586*(7831), 776-778. doi:10.1038/s41586-020-2334-5

Smith, S. L., Anderson, E. R., Cansado-Utrilla, C., Prince, T., Farrell, S., Brant, B., . . . Patterson, E. I. (2021). SARS-CoV-2 neutralising antibodies in dogs and cats in the United Kingdom. *Current Research in Virological Science, 2*, 100011. doi:https://doi.org/10.1016/j.crviro.2021.100011

Stevanovic, V., Tabain, I., Vilibic-Cavlek, T., Mauric Maljkovic, M., Benvin, I., Hruskar, Z., . . . Barbic, L. (2021). The Emergence of SARS-CoV-2 within the Dog Population in Croatia: Host Factors and Clinical Outcome. *Viruses, 13*(8), 1430. Retrieved from https://www.mdpi.com/1999-4915/13/8/1430

Stevanovic, V., Vilibic-Cavlek, T., Tabain, I., Benvin, I., Kovac, S., Hruskar, Z., . . . Barbic, L. (2020). Seroprevalence of SARS-CoV-2 infection among pet animals in Croatia and potential public health impact. *Transbound Emerg Dis, n/a*(n/a). doi:https://doi.org/10.1111/tbed.13924

The European Pet Food Industry. (2020). FACTS & FIGURES 2020 European Overview. doi:https://fediaf.org/images/FEDIAF_Facts_and_Figures_2020.pdf

Udom, K., Jairak, W., Chamsai, E., Charoenkul, K., Boonyapisitsopa, S., Bunpapong, N., . . . Amonsin, A. (2021). Serological survey of antibodies against SARS-CoV-2 in dogs and cats, Thailand. *Transbound Emerg Dis*. doi:10.1111/tbed.14208

Zhang, Q., Zhang, H., Gao, J., Huang, K., Yang, Y., Hui, X., . . . Jin, M. (2020). A serological survey of SARS-CoV-2 in cat in Wuhan. *Emerg Microbes Infect, 9*(1), 2013-2019. doi:10.1080/22221751.2020.1817796

Zhao, S., Schuurman, N., Li, W., Wang, C., Smit, L. A. M., Broens, E., . . . Egberink, H. (2021). Serologic Screening of Severe Acute Respiratory Syndrome Coronavirus 2 Infection in Cats and Dogs during First Coronavirus Disease Wave, the Netherlands. *Emerging Infectious Disease journal, 27*(5), 1362. doi:10.3201/eid2705.204055