

High prevalence of SARS-CoV-2 antibodies in pets from COVID-19+ households

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35 **Abstract**

36 In a survey of household cats and dogs of laboratory-confirmed COVID-19 patients, we found a high
37 seroprevalence of SARS-CoV-2 antibodies, ranging from 21% to 53%, depending on the positivity
38 criteria chosen. Seropositivity was significantly greater among pets from COVID-19+ households
39 compared to those with owners of unknown status. Our results highlight the potential role of pets in
40 the spread of the epidemic.

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42 **Main**

43 Since its emergence in December 2019, in Wuhan, China, Respiratory Syndrome Coronavirus 2 (SARS-
44 CoV-2) has spread throughout the world, probably exclusively through human-to-human
45 transmission. However, the existence of hundreds of millions of companion animals living closely
46 with humans raises the question of their susceptibility to infection and potential role in the outbreak.

47 Cats and dogs are known to be infected by Alphacoronaviruses and Betacoronavirus (Feline CoVs,
48 Canine CoVs)¹, and thus may be susceptible to SARS-CoV-2, which also belongs to the
49 Betacoronavirus group. In Europe, the prevalence of canine coronavirus infection is low². Feline
50 coronavirus prevalence is higher³⁻⁵, with typical seroprevalence ranging from 50% in healthy Swiss
51 cats to 37% in Japan. Additionally, epidemiological, biological, and virological characteristics of
52 coronaviruses, mainly based on Spike-protein plasticity, suggest species barriers to infection may be
53 easily crossed. Thus, pet contamination by sick owners is not only likely but perhaps expected, given
54 the numerous opportunities for spillover⁶⁻⁸. The observation of several cases of mild infections in
55 dogs and cats of infected owners, and serological surveys of pet populations reporting infection rates
56 ranging from 0% to 15,8%⁹⁻¹², highlight this risk. Yet, despite these observations, studies continue to
57 suggest that the risk of contamination of pets by their owners is low and that the role of pets in the
58 spread of the outbreak is trivial or nonexistent.

59 Presently there is no published study accurately assessing the contamination levels in household
60 pets. Here we present results from a serological survey of pets conducted between May and June

61 2020 in two neighbouring regions of eastern France: Franche-Comté and Rhône-Alpes. Both regions
62 had similar epidemiological characteristics and health management policies, with the first
63 hospitalised deaths registered in March 2020 ([https://www.gouvernement.fr/info-coronavirus/carte-
et-donnees](https://www.gouvernement.fr/info-coronavirus/carte-et-donnees)). The first group of pets, from the Franche-Comté region, were living in homes where at
64 least one person expressed respiratory symptoms and tested positive for SARS-CoV-2 at the
65 University Hospital of Besançon (COVID-19+ household group). The second group, from the Rhône-
66 Alpes, were pets from households where exposure was unknown (unknown status household group).
67 Lastly, we included a control group of animals sampled in 2018 and early 2019 before the outbreak,
68 including hyperimmune sera from ten cats with feline infectious peritonitis virus (FIPV), (Control
69 group). Inclusion FIPV-infected cat sera in the control group allows us to exclude possible cross-
70 reactivity of antibodies generated in response to non-SARS-CoV-2 coronaviruses.
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73 We combined four different tests based on two different techniques to ensure the greatest degree of
74 specific-antibody detection. Three microsphere immunoassays (MIA) detected anti-SARS-CoV-2 IgGs
75 produced in response to viral N, S1, or S2 proteins, and a retrovirus-based pseudoparticle assay
76 detected SARS-CoV-2 neutralizing antibodies (Methods). Taking into account these two types of
77 assays, animals were declared COVID-19 positive following a positive seroneutralization assay or if
78 they were positive for all three MIAs. This positivity criterion ensures 100% specificity, as none of
79 the animals in the control group tested positive for the three MIAs or for seroneutralisation (Fig. 1a-
80 d).

81 A remarkably high 21.3% (10 of 47 animals tested) of pets in COVID-19+ households tested positive,
82 including 23.5% of cats (8/34) and 15.4% of dogs (2/13), a non-significant difference ($p=0.70$) (Fig. 1a-
83 e, Supplementary tables 1-2). Of the 16 cats and 22 dogs tested from households of unknown status,
84 only one animal (a cat) tested positive (Fig. 1a-e, Supplementary tables 1-2), representing a
85 significantly lower seroprevalence than the COVID-19+ group ($p=0.0194$). The risk of testing

86 seropositive was eight times higher for pets sharing a home with a COVID-19+ person than for pets in
87 homes of unknown status (relative risk of being seropositive = 8.1).

88

89 Though we cannot definitively prove that all the ten positive animals were infected with SARS-CoV-2,
90 the much greater seroprevalence in animals from COVID-19+ households provides strong evidence
91 that pets have been infected with SARS-CoV-2.

92 The highly variable antibody responses to SARS-CoV-2 in human infections^{13,14}, calls into question our
93 strict criteria for defining seropositive tests. If we consider an animal seropositive if any one test was
94 positive, 53.2% in pets from COVID-19+ households show signs of having been infected (58.8% of
95 cats (20/34) and 38.5% of dogs (5/13)) compared to 15.8% (6/38) of pets in homes of unknown
96 status.

97 A recent Swiss study found that anti-N antibody assays substantially underestimate (i.e., by 30% to
98 45%) the proportion of SARS-CoV-2 exposed individuals compared to anti-S antibody assays in
99 population-based seroprevalence studies¹⁵. Assuming similar dynamics in pets, the actual
100 seropositivity in COVID-19+ households is likely closer to 53% than 21%, indicating that infection risk
101 in the pets of COVID-19 positive owners is much higher than previously described. Given that cats
102 and dogs may become infected, do they contribute to COVID-19 spread due to spillover back into
103 humans? While viral shedding from pets does not appear sufficient for transmission to humans or
104 other animals encountered during walks, for people in closer contact, precautionary measures
105 should be considered as part of a 'one health' global control strategy.

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111 **Methods**

112 The dataset generated during the current study are available from the corresponding authors on
113 reasonable request.

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115 **COVID19+ household group**

116 The COVID19+ household group was recruited from a cohort of 825 patients diagnosed with SARS-
117 CoV-2 infection by reverse-transcriptase–polymerase-chain-reaction testing of nasopharyngeal
118 swabs in the infectious tropical disease department at the University Hospital of Besançon between
119 March 1 to April 25. From May 11 to 22, 384 patients were contacted and 84 reported owning dogs
120 and/or cats. 34 gave us their informed consent to sample their pets. Whole blood samples were
121 collected from 13 dogs and 34 cats between June 7 and June 12, 2 to 3 months after the owners
122 were diagnosed.

123

124 **Unknown status household group**

125 The unknown status household group recruited volunteers among staff and students at VetAgro Sup
126 (Lyon's National Veterinary School). Dogs and cats from all volunteers were included. The COVID-19
127 status of the pet owners was unknown. Blood samples were obtained from each animal (no selection)
128 from 14th of May to 4th of June 2020. Clinical examination at the time of sampling indicated that all
129 animals were healthy. Sampling of animals for this study was approved by VetAgro Sup ethical
130 committee (approval number n°2031).

131

132 **Neutralization activity measurement**

133 To measure the neutralizing activity in sera, we developed a MLV-based pseudoparticle carrying a
134 GFP reporter pseudotyped with SARS-CoV2 spike (SARS-CoV-2pp). Briefly, SARS-CoV-2pp were
135 incubated in 1/100 dilution of sera at 37°C for 1 hour. The mix was added on reporter cells (VeroE6),
136 spinoculated for 2 hours (2.500g, 25°C). After 2 hours of incubation, the inoculum was removed and

137 replaced with fresh medium and cells were incubated for 72h before FACS analysis. The level of
138 infectivity was expressed as % of GFP positive cells and compared to cells infected with SARS-CoV-
139 2pp incubated without serum. Prepandemic (including non SARS-CoV2 coronaviruses positive) sera
140 from France were used as negative controls, and anti-SARS-CoV-2 RBD antibody was used as positive
141 control. Seroneutralization specificity was 100% as already described.

142

143 **Microsphere immunoassay**

144 Dog and cat serum samples were tested using a multiplex Microsphere immunoassay (MIA). 10µg of
145 three recombinant SARS-CoV-2 antigens (nucleoprotein, spike subunit 1 and spike subunit 2) were
146 used to capture specific serum antibodies, whereas a recombinant human protein (O⁶-methylguanine
147 DNA methyltransferase) was used as a control antigen in the assay. Distinct MagPlex microsphere
148 sets (Luminex Corp) were respectively coupled to viral or control antigens using the amine coupling
149 kit (Bio-Rad Laboratories) according to manufacturers' instructions. This three microsphere
150 immunoassays (MIA) were developed and provided by Institut Pasteur, Paris. The MIA procedure was
151 performed by incubating the serum samples (50 µl), diluted 1:400 in assay buffer (PBS-1% BSA-0.05%
152 Tween 20), with the mixture of antigen-coated bead sets (about 1250 beads of each type) protected
153 from the light on an orbital shaker at 700 rpm for 30 min. After washing, 50 µl of biotinylated protein
154 A and biotinylated protein G (Thermo Fisher Scientific) at a 4 µg/ml each in assay buffer were
155 transferred to each well and incubated on an orbital shaker for 30 min at 700 rpm in the dark. After
156 washing, the beads were incubated for 10min at 700 rpm in the dark with 50 µl of Streptavidin-R-
157 Phycoerythrin (Life technologies) diluted to 4 µg/mL in assay buffer. After washing, beads were
158 resuspended in 100 µl of assay buffer. Measurements were performed using a Magpix instrument
159 (Luminex), at least 100 events were read for each bead set and binding events were displayed as
160 median fluorescence intensities (MFI). Relative Fluorescence Intensities (RFI) were calculated for
161 each sample by dividing the MFI signal measured for the antigen-coated microsphere sets by the MFI
162 signal obtained for the control microsphere set, to account for nonspecific binding of antibodies to

163 beads. Specific seropositivity cut-off values for each antigen were set at three standard deviations
164 above the mean RFI of the 37 dog (from France and Gabon) and 14 cat samples (from France) from
165 the control group sampled before 2019. Based on the prepandemic population, MIA specificity was
166 set at 97,3% for dogs and 100% for cats.

167

168 **Statistical analyses**

169 Fisher's exact test was used to analyze differences in antibody detection between the COVID19+
170 household group and the unknown status household group, as well as tests comparing cats and dogs
171 in COVID-19+ households.

172

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189 **Author contributions**

190 V.L. and E.L. conceived the study.

191 M.F., B.R., E.K., P.B., E.R., O.V., S.D., B.B., J.V., A.K., V.L. and E.L. designed and performed the
192 experiments.

193 M.F., B.R., E.K., A.K., V.L., E.L. designed the work

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

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

196

197 **Competing Interests**

198 The authors declare no competing interests.

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241 **Figure Legend**

242 **Figure 1. High prevalence of SARS-CoV-2 antibodies in COVID19+ household pets**

243 Serological evaluation of anti-SARS-CoV-2 antibodies in pets from unknown status and COVID19+
244 households. COVID19+ households had at least one COVID-19 laboratory-confirmed person (Green).
245 Unknown status households were those with no confirmed SARS-CoV-2 infected person (Black).
246 Control include pre-pandemic population (Grey) and FIPV infected cats (Brown). **a**: Anti-N antibody
247 levels. **b**: Anti-S1 antibody levels. **c**: Anti-S2 antibody levels. SARS-CoV-2 specific antibody levels were
248 assessed using MIAs and expressed as Relative Fluorescence Intensities (RFI) to control antigen. A pre-
249 pandemic population was used to determine the cut-off (mean + 3*standard deviation). **d**: Percentage
250 of neutralizing activity in pet sera. Neutralising activity was assessed using a pseudoparticle assay and
251 expressed as the percent neutralization relative to a no serum condition. For **a,b,c,d** mean line are
252 presented. **e**: Prevalence based on positive anti-N, anti-S1, anti-S2, and seroneutralization tests in
253 COVID19+ and unknown status households. 95% confidence interval are presented (\pm 95% confidence
254 intervals).

