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

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Abstract

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

Main

Since its emergence in December 2019, in Wuhan, China, Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has spread throughout the world, probably exclusively through human-to-human transmission. However, the existence of hundreds of millions of companion animals living closely with humans raises the question of their susceptibility to infection and potential role in the outbreak. Cats and dogs are known to be infected by Alphacoronaviruses and Betacoronavirus (Feline CoVs, Canine CoVs)[1], and thus may be susceptible to SARS-CoV-2, which also belongs to the Betacoronavirus group. In Europe, the prevalence of canine coronavirus infection is low[2]. Feline coronavirus prevalence is higher[3-5], with typical seroprevalence ranging from 50% in healthy Swiss cats to 37% in Japan. Additionally, epidemiological, biological, and virological characteristics of coronaviruses, mainly based on Spike-protein plasticity, suggest species barriers to infection may be easily crossed. Thus, pet contamination by sick owners is not only likely but perhaps expected, given the numerous opportunities for spillover[6-8]. The observation of several cases of mild infections in dogs and cats of infected owners, and serological surveys of pet populations reporting infection rates ranging from 0% to 15,8% [9-12], highlight this risk. Yet, despite these observations, studies continue to suggest that the risk of contamination of pets by their owners is low and that the role of pets in the spread of the outbreak is trivial or nonexistent.

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

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61   2020 in two neighbouring regions of eastern France: Franche-Comté and Rhone-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-

64   et-donnees). The first group of pets, from the Franche-Comté region, were living in homes where at

65   least one person expressed respiratory symptoms and tested positive for SARS-CoV-2 at the

66   University Hospital of Besançon (COVID-19+ household group). The second group, from the Rhone-

67   Alpes, were pets from households where exposure was unknown (unknown status household group).

68   Lastly, we included a control group of animals sampled in 2018 and early 2019 before the outbreak,

69   including hyperimmune sera from ten cats with feline infectious peritonitis virus (FIPV), (Control

70   group). Inclusion FIPV-infected cat sera in the control group allows us to exclude possible cross-

71   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 MIA tests. 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

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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).

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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 studies[15]. 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).

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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.

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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 (O^6-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

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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.

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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.

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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.

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197 **Competing Interests**

198 The authors declare no competing interests.

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200 **References**

201

202 1.   Le Poder, S. Feline and canine coronaviruses: common genetic and pathobiological features.

203   *Advances in virology* **2011**, 609465 (2011).

204 2.   Stavisky, J*., et al.* Prevalence of canine enteric coronavirus in a cross-sectional survey of dogs

205   presenting at veterinary practices. *Vet Microbiol* **140**, 18-24 (2010).

206 3.   Bell, E.T., Toribio, J.A., White, J.D., Malik, R. & Norris, J.M. Seroprevalence study of feline

207   coronavirus in owned and feral cats in Sydney, Australia. *Australian veterinary journal* **84**, 74-

208   81 (2006).

209 4.   Kummrow, M*., et al.* Feline coronavirus serotypes 1 and 2: seroprevalence and association

210   with disease in Switzerland. *Clin Diagn Lab Immunol* **12**, 1209-1215 (2005).

211 5.   Taharaguchi, S., Soma, T. & Hara, M. Prevalence of feline coronavirus antibodies in Japanese

212   domestic cats during the past decade. *J Vet Med Sci* **74**, 1355-1358 (2012).

213 6.   Andersen, K.G., Rambaut, A., Lipkin, W.I., Holmes, E.C. & Garry, R.F. The proximal origin of

214   SARS-CoV-2. *Nature Medicine* **26**, 450-452 (2020).

8

215 7. Leroy, E.M., Ar Gouilh, M. & Brugère-Picoux, J. The risk of SARS-CoV-2 transmission to pets

216   and other wild and domestic animals strongly mandates a one-health strategy to control the

217   COVID-19 pandemic. *One Health*, 100133-100133 (2020).

218 8. Wan, Y., Shang, J., Graham, R., Baric, R.S. & Li, F. Receptor Recognition by the Novel

219   Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS

220   Coronavirus. *Journal of Virology* **94**, e00127-00120 (2020).

221 9. Chen, J., Huang, C., Zhang, Y., Zhang, S. & Jin, M. Severe Acute Respiratory Syndrome

222   Coronavirus 2-Specific Antibodies in Pets in Wuhan, China. *The Journal of infection* (2020).

223 10. Deng, J.*, et al.* Serological survey of SARS-CoV-2 for experimental, domestic, companion and

224   wild animals excludes intermediate hosts of 35 different species of animals. *Transboundary*

225   *and emerging diseases* (2020).

226 11. Sailleau, C.*, et al.* First detection and genome sequencing of SARS-CoV-2 in an infected cat in

227   France. *Transboundary and emerging diseases* (2020).

228 12. Sit, T.H.C.*, et al.* Infection of dogs with SARS-CoV-2. *Nature* (2020).

229 13. Grzelak, L.*, et al.* A comparison of four serological assays for detecting anti-SARS-CoV-2

230   antibodies in human serum samples from different populations. *Science translational*

231   *medicine* (2020).

232 14. Okba, N.M.A.*, et al.* Severe Acute Respiratory Syndrome Coronavirus 2–Specific Antibody

233   Responses in Coronavirus Disease Patients. *Emerging Infectious Disease journal* **26**, 1478

234   (2020).

235 15. Fenwick, C.*, et al.* Changes in SARS-CoV-2 Antibody Responses Impact the Estimates of

236   Infections in Population-Based Seroprevalence Studies. *medRxiv*, 2020.2007.2014.20153536

237   (2020).

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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 (± 95% confidence

254 intervals).