

1 SARS-CoV-2 neutralising antibodies in Dogs and Cats in the United Kingdom

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25 Abstract

26 Companion animals are susceptible to SARS-CoV-2 infection and sporadic cases of pet  
27 infections have occurred in the United Kingdom. Here we present the first large-scale  
28 serological survey of SARS-CoV-2 neutralising antibodies in dogs and cats in the UK. Results  
29 are reported for 688 sera (454 canine, 234 feline) collected by a large veterinary diagnostic  
30 laboratory for routine haematology during three time periods; pre-COVID-19 (January  
31 2020), during the first wave of UK human infections (April-May 2020) and during the second  
32 wave of UK human infections (September 2020-February 2021). Both pre-COVID-19 sera  
33 and those from the first wave tested negative. However, in sera collected during the second  
34 wave, 1.4% (n=4) of dogs and 2.2% (n=2) cats tested positive for neutralising antibodies. The  
35 low numbers of animals testing positive suggests pet animals are unlikely to be a major  
36 reservoir for human infection in the UK. However, continued surveillance of in-contact  
37 susceptible animals should be performed as part of ongoing population health surveillance  
38 initiatives.

39

40 Key words

41 SARS-CoV-2, serology, dogs, cats, animal disease surveillance

42

43 Introduction

44 Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged in Wuhan, China at  
45 the end of 2019 [1] and rapidly spread around the world. The main route of transmission  
46 remains human-to-human. However, there is evidence that the virus can infect animals [2]  
47 and it is important that we remain vigilant of such infections; particularly in companion  
48 animals with whom humans often have close contact.

49

50 Although initially there were only sporadic cases of infection in cats and dogs [3-5], there  
51 are now numerous reports of infection detected by RT-PCR or virus isolation [6-10],  
52 including in the UK [11]. Evidence of infection of cats and dogs has also been provided by  
53 the detection of anti-SARS-CoV-2 antibodies in several studies; from Italy, France, Germany,  
54 Croatia and China [12-17]. Experimental infections have shown that cats and, to a lesser  
55 extent, dogs are susceptible to SARS-CoV-2 and that cats can transmit the virus to other cats  
56 [18-20]. Infections in companion animals appear to have occurred as a result of human-to-  
57 animal transmission; however, the reported transmission of SARS-CoV-2 from farmed mink  
58 to in-contact humans, cats and dogs [21, 22] and the detection of the virus in stray dogs and  
59 cats [23, 24], suggest it is important to continue surveillance in companion animals. Here we  
60 conducted a survey of SARS-CoV-2 neutralising antibodies in cats and dogs attending UK  
61 veterinary practices.

62

63 Methods

64 Samples

65 Canine and feline sera used in this study were obtained from the UK Virtual Biobank, which  
66 uses health data from commercial diagnostic laboratories participating in the Small Animal  
67 Veterinary Surveillance Network (SAVSNET) to target left over diagnostic samples in the  
68 same laboratories for enhanced phenotypic and genomic analyses [25]. All samples were  
69 residual sera remaining after routine diagnostic testing and were sent by the contributing  
70 laboratory based on convenience within the following parameters: samples were requested  
71 from UK cats and dogs collected over two time periods; March and April 2020 (early  
72 pandemic) for both cats and dogs, then September 2020 to February 2021 for dogs, and

73 January 2021 for cats (late pandemic). Serum samples collected from the same laboratory in  
74 early January 2020 were also tested as pre-COVID-19 controls. All samples were linked to  
75 electronic health data for that sample (species, breed, sex, postcode of the submitting  
76 veterinary practice, date received by the diagnostic laboratory) held in the SAVSNET  
77 database, using a unique anonymised identifier. Data on SARS-CoV-2 exposure or symptoms  
78 was not available. Ethical approval to collect electronic health data (SAVSNET) and physical  
79 samples from participating laboratories (National Virtual Biobank) was granted by the  
80 Research Ethics Committee at the University of Liverpool (RETH000964).

81

82 Neutralising antibody detection in serum samples  
83 Serum samples were screened for SARS-CoV-2 neutralising antibodies using the plaque  
84 reduction neutralisation test (PRNT) as previously described [15], with the SARS-CoV-  
85 2/human/Liverpool/REMRQ0001/2020 isolate cultured in Vero E6 cells [26]. Briefly, sera  
86 were heat inactivated at 56°C for 30 mins and stored at -20°C until use. DMEM containing  
87 2% FBS was used to dilute sera ten-fold followed by serial two-fold dilution. SARS-CoV-2 at  
88 800 plaque forming units (PFU)/ml was added to diluted sera and incubated at 37°C for 1 h.  
89 The virus/serum mixture was then inoculated onto Vero E6 cells, incubated at 37°C for 1 h,  
90 and overlaid as in standard plaque assays [27]. Cells were incubated for 48 h at 37°C and 5%  
91 CO<sub>2</sub>, fixed with 10% formalin and stained with 0.05% crystal violet solution. PRNT<sub>80</sub> was  
92 determined by the highest dilution with 80% reduction in plaques compared to the control.  
93 Samples with detectable neutralising antibody titre were repeated as technical replicates  
94 for confirmation. Where titres differed between technical replicates, the lowest dilution was  
95 reported.

96

97 Results

98 A total of 732 samples were received from the diagnostic laboratory and tested for SARS-  
99 CoV-2 neutralising antibodies. Linking of data to the samples found that 22 samples were  
100 duplicates (duplicate samples gave the same result in each replicate and are therefore  
101 reported as one sample). Seven samples were from animals with non-UK postcodes, two  
102 samples did not have species data, two samples were received as dogs but were actually  
103 from cats and were collected outside the two time periods of cat sample collection and  
104 eleven samples were missing postcodes; these samples were excluded. Results are  
105 therefore reported for 688 sera (454 canine, 234 feline) of which 558 (372 dogs, 186 cats)  
106 were collected during the SARS-CoV-2 pandemic and 130 (82 dogs, 48 cats) were collected  
107 from animals before the first confirmed human case in the UK (21<sup>st</sup> January 2020 [28]) - pre-  
108 COVID-19 samples; these samples were distributed across the UK (Figure 1). Of the dog sera  
109 collected during the pandemic, 0/85 (0%) collected in March/April 2020 and 4/287 (1.4%)  
110 collected September 2020-February 2021 tested positive for neutralising antibodies with  
111 titres ranging from 1:20 to 1:80. In cats, 0/96 (0%) sera collected in March/April 2020 tested  
112 positive for neutralising antibodies and 2/90 (2.2%) collected in January 2021 tested positive  
113 with titres of 1:40 and 1:80. Pre-COVID-19 sera from both dogs (n=82) and cats (n=48)  
114 tested negative for neutralising antibodies. Positive samples in dogs were collected in  
115 November 2020 (n=1), January 2021 (n=2) and February 2021 (n=1) and were collected in  
116 Kent, Buckinghamshire, Worcestershire and Yorkshire, respectively (Figure 1). The two  
117 positive cats were collected in January 2021; one in Birmingham and the other in London  
118 (Figure 1).

119

120 Discussion

121 SARS-CoV-2 emerged in humans in China late in 2019, rapidly spreading across the world.  
122 Studies of companion animals from several countries have shown that they too can be  
123 infected with the virus. In the UK, there are sporadic reports of infection in cats and dogs  
124 [11, 29], however, there has been no large scale test of infection. Here we show that a small  
125 proportion of UK dogs and cats sampled at a time of active human transmission tested  
126 positive for SARS-CoV-2 neutralising antibodies.

127  
128 Sera from two time points during the pandemic were analysed. Sera collected early in the  
129 pandemic, during March and April 2020, from both cats and dogs were negative for  
130 neutralising antibodies. Previous studies using European samples have shown a low level of  
131 infection, highest in Italy, where 3.3% (15/451) of dog sera and 5.8% (11/191) cat sera  
132 collected between March and May 2020 had measurable neutralising antibody titres [15].  
133 These samples were purposefully collected from regions of Italy with a high prevalence of  
134 infection in humans, in some cases from households known to contain recently diagnosed  
135 human cases. Our results in contrast, are more consistent with a survey from a similar  
136 population of cats in Germany, that found 0/221 samples collected in April and May of 2020  
137 to be positive for anti-SARS-CoV-2 antibodies using ELISA [13], and with a survey in the  
138 Netherlands in April-May 2020, that found 0.4% of cats and 0.2% dogs to be seropositive  
139 [30]. Lack of positive samples from this time period in the UK (April-May2020) likely reflects  
140 the selection criteria of the animals assayed (undergoing routine haematological testing and  
141 not selected based on location), and the relatively low rate of human disease at the time  
142 compared to Italy.

143

144 In sera collected later in the pandemic, 4/287 (1.4%) dogs and 2/90 (2.2%) cats tested  
145 positive. Positive dog samples were collected in November 2020 and January and February  
146 of 2021. Positive cats were collected in January 2021. This is again broadly in line with a  
147 recent German survey conducted from September 2020 to February 2021, showing a  
148 seroprevalence of 1.36%, that the authors concluded corresponded with the rise of  
149 reported cases in the human population, and was suggestive of ongoing transmission from  
150 owners to their cats [14].

151

152 Cats and dogs can be infected with other coronaviruses, leading to the possibility that SARS-  
153 CoV-2 neutralising antibodies in cats and dogs may result from previous infection with a  
154 different virus. We and others have previously demonstrated a lack of cross-reactivity  
155 between SARS-CoV-2 and samples containing antibodies to feline coronavirus (FCoV), canine  
156 enteric coronavirus (CeCoV) and canine respiratory coronavirus (CRCoV) [13, 15, 16]; all of  
157 which are endemic in UK cats and dogs [31-33]. Here we also tested samples from UK cats  
158 and dogs collected before the human index case in the UK (21<sup>st</sup> January 2020 [28]). All pre-  
159 COVID-19 samples were negative for SARS-CoV-2 neutralising antibodies. Similar results  
160 have been reported for both cats and dogs by others [30], suggesting that antibodies  
161 produced following infection by cat and dog coronaviruses do not cross react with SARS-  
162 CoV-2.

163

164 Here we made use of samples collected from a commercial diagnostic laboratory  
165 contributing data to a voluntary national surveillance scheme (SAVSNET) to efficiently test  
166 for evidence of prior SARS-CoV-2 infection in UK cats and dogs. The major limitations of such  
167 a system are the relatively sparse data available for each sample such that individual

168 animals, that are not identifiable, may have been sampled twice or have come from the  
169 same household. In addition, such samples lack detailed information on the health of the  
170 animals and whether they were from a COVID-19-positive household. However, acquiring  
171 such samples from the UK Virtual Biobank, offers a responsive resource for studying  
172 national patterns of disease in UK pets [25].

173

174 We report here the detection of SARS-CoV-2 neutralising antibodies during the second wave  
175 of human infections in the UK. Other groups have previously reported that cats and dogs  
176 can become infected, likely through their interactions with humans. Although animal-to-  
177 animal transmission has been reported, for example on mink farms and in experimental  
178 infections [18-20, 22, 34], the small numbers of companion animals testing positive in the  
179 field suggest that pets are not currently acting as a significant reservoir for infection, and  
180 that the pandemic will be controlled by measures largely focussed on minimising human-to-  
181 human transmission. However, studies like that presented here strongly argue for continued  
182 surveillance of in-contact, susceptible animal species, which will help determine whether in  
183 the future, more targeted control measures are needed for pet animals, particularly in  
184 regions that are gaining control of infection in their human populations.

185

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199

200 Conflict of interest

201 NM and LR are employed by IDEXX Laboratories. All other authors declare no competing  
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288

289 Figure 1: Schematic map showing the location of samples for which testing of SARS-CoV-2  
290 neutralising antibodies is reported. Red dots indicate samples that were positive for SARS-  
291 CoV-2 neutralising antibodies using PRNT<sub>80</sub>. Blue dots indicate samples that were negative.