

1 **Evidence of exposure to SARS-CoV-2 in cats and dogs from households in**
2 **Italy**

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29

30 **Abstract:** SARS-CoV-2 originated in animals and is now easily transmitted between people.
31 Sporadic detection of natural cases in animals alongside successful experimental infections of
32 pets, such as cats, ferrets and dogs, raises questions about the susceptibility of animals under
33 natural conditions of pet ownership. Here we report a large-scale study to assess SARS-CoV-2
34 infection in 817 companion animals living in northern Italy, sampled at a time of frequent human
35 infection. No animals tested PCR positive. However, 3.4% of dogs and 3.9% of cats had
36 measurable SARS-CoV-2 neutralizing antibody titers, with dogs from COVID-19 positive
37 households being significantly more likely to test positive than those from COVID-19 negative
38 households. Understanding risk factors associated with this and their potential to infect other
39 species requires urgent investigation.

40 **One Sentence Summary:** SARS-CoV-2 antibodies in pets from Italy.

41 **Main Text:** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late
42 December 2019 in Wuhan, Hubei province, China (1), possibly as a spillover from bats to

43 humans (2), and rapidly spread worldwide becoming a pandemic (3). Although the virus is
44 believed to spread almost exclusively by human-to-human transmission, there are concerns that
45 some animal species may contribute to the ongoing SARS-CoV-2 pandemic epidemiology (4).
46 To date, sporadic cases of SARS-CoV-2 infection have been reported in dogs and cats. These
47 include detection of SARS-CoV-2 RNA in respiratory and/or fecal specimens of dogs and cats
48 with or without clinical signs (5-7), as well as of specific antibodies in sera from pets from
49 coronavirus disease 2019 (COVID-19) affected areas (7,8). In addition, experimental infection of
50 various animal species has demonstrated that while dogs appear poorly susceptible to SARS-
51 CoV-2 infection, developing asymptomatic infections and shedding low-titer or no virus, cats
52 develop respiratory pathology and shed high titers of SARS-CoV-2, even being able to infect in-
53 contact animals (9,10). Wide scale testing of susceptible species is needed to assess the extent of
54 animal infection under more natural conditions of husbandry. Here, we conducted an extensive
55 epidemiological survey from March to May 2020 in cats and dogs living in Italy, either in
56 SARS-CoV-2 positive households or living in geographic areas that were severely affected by
57 COVID-19. To our knowledge, this is the largest study to investigate SARS-CoV-2 in
58 companion animals to date.

59 All animals were sampled by their private veterinary surgeon during routine healthcare visits.
60 Sampling of animals for this study was approved by the Ethics Committee of the Department of
61 Veterinary Medicine, University of Bari, Italy (approval number 15/2020). A total of 540 dogs
62 and 277 cats were sampled from different Italian regions, mostly Lombardy (476 dogs, 187 cats).
63 Animals were sampled either from regions severely affected by COVID-19 outbreaks in humans
64 or from those that offered convenient access to samples. Oropharyngeal (306 dogs, 175 cats),
65 nasal (185 dogs, 77 cats), and/or rectal (66 dogs, 30 cats) swabs were collected from the sampled

66 pets. For 340 dogs and 188 cats, full signalment and clinical history were available, including
67 breed, sex, age, exposure to COVID-19 infected humans (COVID-19 positive household,
68 suspected COVID-19 positive household but not confirmed by specific assay, and COVID-19
69 negative household), presence of respiratory signs (cough, sneezing, conjunctivitis, nasal and/or
70 ocular discharge).

71 Sera were available for 188 dogs and 63 cats for which complete signalment, history and location
72 were available (Fig. 1). Additional sera were collected from diagnostic laboratories for 200 dogs
73 and 89 cats from the affected areas, but which lacked further historical information.

74 Detection of SARS-CoV-2 RNA used two real-time RT-PCR assays targeting nucleoprotein and
75 envelope protein genes as previously described (11). Plaque reduction neutralization tests
76 (PRNT) were using a previously established protocol (8) with SARS-CoV-
77 2/human/Liverpool/REMRQ0001/2020 isolate was cultured as previously described (9).
78 PRNT80 was determined by the highest dilution with \geq 80% reduction in plaques compared to
79 the control.

80 All of 839 collected swab samples tested negative for SARS-CoV-2 RNA, including 38 cats and
81 38 dogs that showed respiratory symptoms at the time of sampling, suggesting absence of active
82 SARS-CoV-2 infection in the tested animals. In addition, 64 of these dogs and 57 of the cats that
83 tested negative were living in households previously confirmed as having had COVID-19.

84 SARS-CoV-2 neutralizing antibodies were detected in 13 dogs (3.35%) and 6 cats (3.95%), with
85 titers ranging from 1:20 to 1:160 and from 1:40 to 1:1280 in dogs and cats, respectively. Of
86 samples from households with known COVID-19 status, neutralizing antibodies were detected in
87 6 of 47 dogs (12.8%) and 1 of 22 cats (4.5%) from COVID-19 positive households, 1 of 7 dogs
88 (14.3%) and 0 of 3 cats (0%) from suspected COVID-19 positive households and 2 of 133 dogs

89 (1.5%) and 1 of 38 cats (2.6%) from COVID-19 negative households (Table 1). For those 423
90 animals where an age was recorded, 0 of 30 aged less than 1 year (0%), 6 of 92 aged 1-3 years
91 (6.5%), 3 of 102 aged 4-7 years (2.9%) and 6 of 199 aged 8 and over (3.0%) tested positive.
92 None of the animals with neutralizing antibodies displayed respiratory symptoms at the time of
93 sampling.

94 Reference sera or ascitic fluids from animals previously shown to be positive for canine enteric
95 coronavirus (14), canine respiratory coronavirus (15) and feline coronavirus (16) tested negative
96 by the PRNT assay for SARS-CoV-2, confirming the specificity of the obtained results (8).

97 Dogs were significantly more likely to test positive for SARS-CoV-2 neutralizing antibodies if
98 they came from a known COVID-19 positive household (Fisher's exact test, $p=0.004$) or were
99 male (Fisher's exact test, $p=0.045$). In provinces where at least 10 samples were available, there
100 was a strong positive trend between the proportion of dogs that tested positive and the recorded
101 burden of human disease (Spearman's $r = 0.732$, $p = 0.051$) (Fig. 2). A similar association was
102 observed for cats but should be viewed with caution as only four provinces met the criteria for
103 analysis.

104 Following its original probable transmission to humans from animals, SARS-CoV-2 has spread
105 globally within the human population with devastating health and economic impacts. To date,
106 SARS-CoV-2 has been sporadically detected in naturally infected dogs and cats, most of which
107 were living in close contact with infected humans. Most studies of companion animals are small
108 in nature, likely because of an inevitable research focus on human disease. Our results from this
109 extensive study of SARS-CoV-2 infection in owned cats and dogs living in areas where viral
110 transmission was active in the human population demonstrate that both cats and dogs can
111 seroconvert under the normal conditions of pet ownership, and where the burdens of disease are

112 highest in humans.

113 The link between SARS-CoV-2 household infection and a pet's seropositivity was only apparent
114 for dogs, possibly suggesting greater interaction between positive people and their household
115 dogs as compared to cats. This contrasts experimental studies where dogs were less susceptible
116 to infection (9). In addition, a higher proportion of male dogs were seropositive compared to
117 female dogs. Future studies in animals and humans should investigate whether this phenomenon
118 is based in physiological or behavioral differences between males and females. Although there
119 are clear gender differences in outcomes in human COVID-19 infections, with males at higher
120 risk of severe disease, there seems to be no evidence for a difference in infection risk (17). None
121 of the 30 juvenile animals, less than one year-of-age, tested positive. Our findings are consistent
122 with reports of other seropositive naturally exposed cats and dogs which were all adult (6, 7).
123 These findings support use of older animals in experimental infections, which are currently
124 performed on animals less than one year-of-age (9) and may therefore underestimate SARS-
125 CoV-2 susceptibility.

126 In contrast to the serology results, all animals tested negative by PCR, including those animals
127 living in households with confirmed COVID-19 human infection and those with and without
128 respiratory symptoms. This suggests that whilst pet animals can seroconvert, they may shed virus
129 for relatively short periods of time. In experimental studies, cats stopped shedding virus by 10
130 days post infection (dpi) and developed neutralizing antibody responses by 13 dpi (9). Similar
131 results were reported in experimental infection of dogs, in which virus was detected in faeces up
132 to 6 dpi, but not in oropharyngeal swabs (6). However, in a naturally infected Pomeranian dog
133 SARS-CoV-2 RNA was detected from nasal swabs by quantitative RT-PCR for at least 13 days
134 at low titer, whilst the virus was not detected in faecal/rectal samples (7), suggesting that virus

135 shedding patterns may vary in some animals. Half of the challenged dogs had detectable
136 antibodies by 14 dpi. These studies and our own highlight similar challenges in detecting SARS-
137 CoV-2 infection that exist for both humans and animals (18). It is not possible with our field data
138 set to estimate the time of infection in animals that were seropositive, and restrictions on human
139 and animal movement during the pandemic may have delayed visits to veterinary practitioners
140 where sampling occurred. We advocate the inclusion of pets in ongoing assessments of
141 community and household shedding to improve detection of active infection.

142 In this extensive epidemiological survey of SARS-CoV-2, we found that companion animals
143 living in areas of high human infection can become infected. Our results suggest that dogs
144 warrant further investigation regarding SARS-CoV-2 susceptibility in contrast to experimental
145 studies which suggested cats were most susceptible (9). We also observed seropositivity rates in
146 animals comparable to those of humans via community sampling at a similar time in European
147 countries (19-21). This suggests that infection in companion animals is not unusual. Based on
148 current knowledge, it is unlikely that infected pets play an active role in SARS-CoV-2
149 transmission to humans. However, animal-to-human transmission may be more likely under
150 certain environmental conditions, such as the high animal population densities encountered on
151 infected mink farms (22). As and when human transmission becomes rarer and contact tracing
152 becomes more accessible, serological surveillance of pets may be advocated to develop a
153 wholistic picture of community disease dynamics and ensure that all transmission opportunities
154 are terminated.

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259 **Author contributions:** EIP and ND designed the study and wrote the first draft of the
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263 Authors declare no competing interests; and **Data and materials availability:** All data is
264 available in the main text or the supplementary materials.

265 **Fig. 1.** Distribution of dog and cat samples assayed for neutralizing antibody titer across Italy
266 and the region of Lombardy. Data on human COVID-19 cases from the Italian Department of
267 Civil Protection as of May 31, 2020 and population data from the Italian National Institute of
268 Statistics (ISTAT), January 2019.

269 **Fig. 2.** Correlation of percentage of seropositive animals per province and human COVID-19
 270 infection density. Data points were taken from provinces with at least 10 samples. Spearman's
 271 correlation was used to assess association.

272 **Table 1.** Seropositivity among dogs and cats, split into risk factor groupings where data was
 273 available. For household and sex, p value determined by Fisher's exact test. Household COVID
 274 + defined as one or more members of a household with a confirmed positive COVID-19 test. All
 275 the information was not available for all the animals.

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Risk factor	Dogs			Cats		
	No. + (total)	%	<i>p</i>	No. + (total)	%	<i>p</i>
Household			0.004			>0.999
COVID+	6 (47)	12.8%		1 (22)	4.5%	
COVID-	2 (133)	1.5%		1 (38)	2.6%	
Sex			0.045			0.492
Male	7 (83)	8.4%		2 (31)	6.5%	
Female	2 (105)	1.9%		0 (30)	0.0%	
Age (years)			na			na
< 1	0 (20)	0.0%		0 (9)	0.0%	
1-3	5 (70)	7.1%		1 (22)	4.5%	
4-7	2 (83)	2.4%		1 (19)	5.3%	
8+	4 (137)	2.9%		2 (62)	3.2%	

Unknown	2 (78)	2.6%	2 (39)	5.1%
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Materials and Methods

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Samples

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All animals were sampled by their private veterinary surgeon during a healthcare visit for other reasons. A total of 540 dogs and 277 cats were sampled from different Italian regions, mostly Lombardy (476 dogs, 187 cats). Animals were sampled either from regions severely affected by COVID-19 outbreaks in humans or from those that offered convenient access to samples.

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Oropharyngeal (306 dogs, 175 cats), nasal (185 dogs, 77 cats), and/or rectal (66 dogs, 30 cats) swabs were collected from the sampled pets. For 340 dogs and 188 cats, full signalment and clinical history were available, including breed, sex, age, exposure to COVID-19 infected humans (COVID-19 positive household, suspected COVID-19 positive household but not confirmed by specific assay, and COVID-19 negative household), presence of respiratory signs (cough, sneezing, conjunctivitis, nasal and/or ocular discharge).

291

Sera were available for 188 dogs and 63 cats for which complete signalment, history and location were available (Figure 1). Additional sera were collected from diagnostic laboratories for 200 dogs and 89 cats from the affected areas, but which lacked further historical information.

294

Sampling of animals for this study was approved by the Ethics Committee of the Department of Veterinary Medicine, University of Bari, Italy (approval number 15/2020).

296

297 *Polymerase chain reaction*

298 Sample preparation and RNA extraction were carried out in the biosafety level 3 containment
299 laboratory at the Department of Veterinary Medicine, University of Bari, Italy. Detection of
300 SARS-CoV-2 RNA used two real-time RT-PCR assays targeting nucleoprotein and envelope
301 protein genes as previously described (11).

302

303 *Plaque reduction neutralization test (PRNT)*

304 The SARS-CoV-2/human/Liverpool/REMRQ0001/2020 isolate was cultured in Vero E6 cells as previously
305 described (12). PRNTs were performed as previously described (13). Briefly, sera were heat inactivated at 56°C for
306 1 hour and stored at -20°C until use. Dulbecco's minimal essential medium (DMEM) containing 2% fetal bovine
307 serum (FBS) and 0.05 mg/mL gentamicin was used for serial two-fold dilutions of serum. SARS-CoV-2 at 800
308 PFU/mL was added to an equal volume of diluted serum and incubated at 37°C for 1 hour. The virus-serum dilution
309 was inoculated onto Vero E6 cells, incubated at 37°C for 1 hour, and overlaid as in standard plaque assays. Cells
310 were incubated for 48 hours at 37°C and 5% CO₂ then fixed with 10% formalin and stained with 0.05% crystal
311 violet solution. PRNT₈₀ was determined by the highest dilution with 80% reduction in plaques compared to the
312 control. Samples with detectable neutralizing antibody titer were repeated as technical replicates for confirmation.

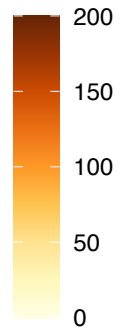
313

314 *Data analysis*

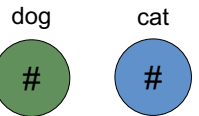
315 Fisher's exact test was used to analyze differences in antibody detection from households with known COVID-19
316 infection status, and antibody detection from male and female animals. Spearman correlation was used to analyze
317 the relationship between human COVID-19 case numbers and detection of antibodies in animals. All statistical
318 analyses were performed in GraphPad Prism.

319

Human COVID-19 cases per 10,000 (as of 31 May 2020)

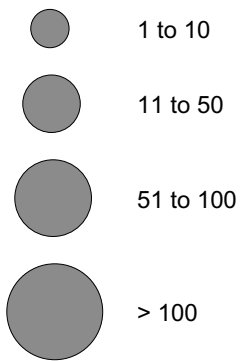


Animal

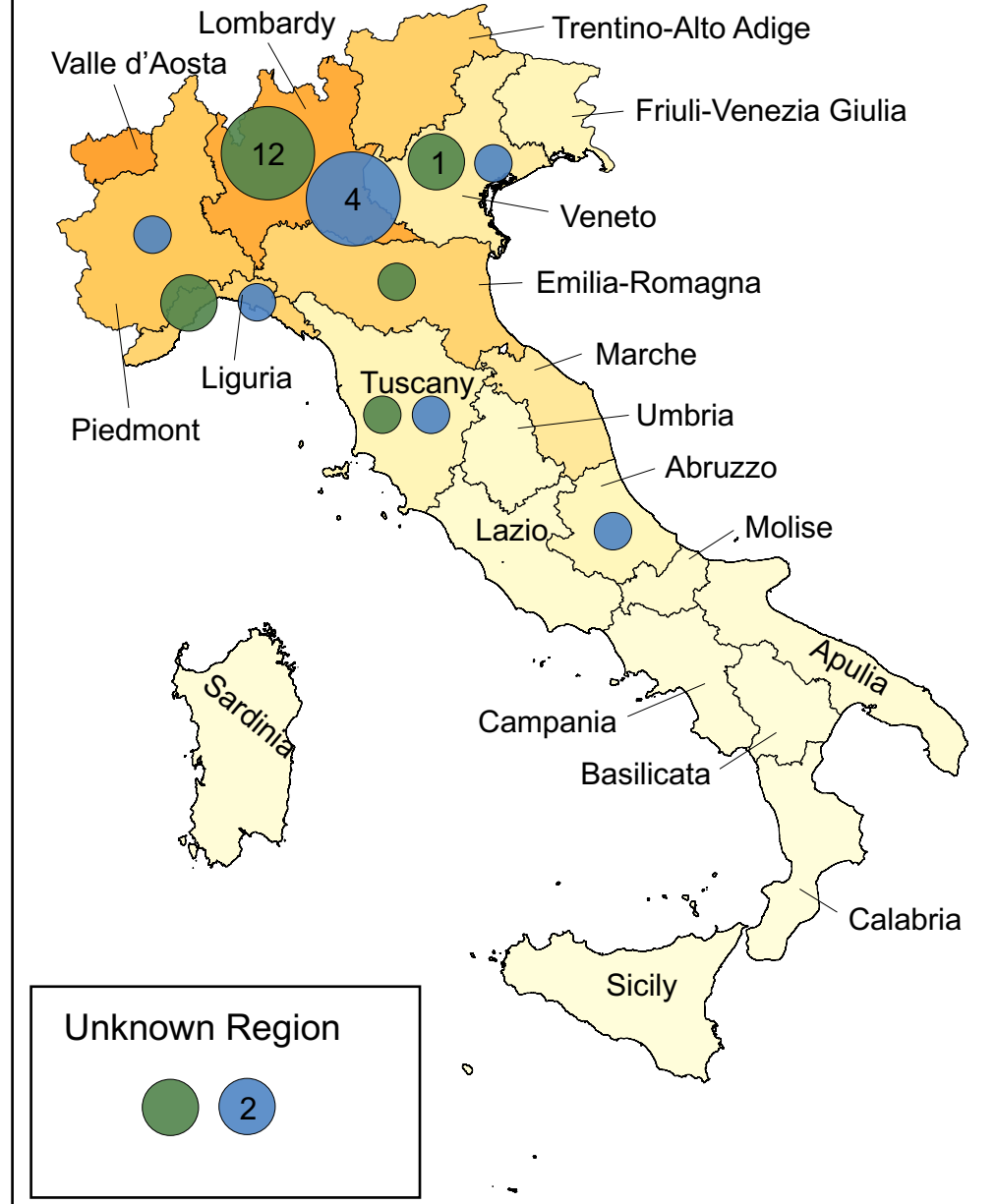


= number positive

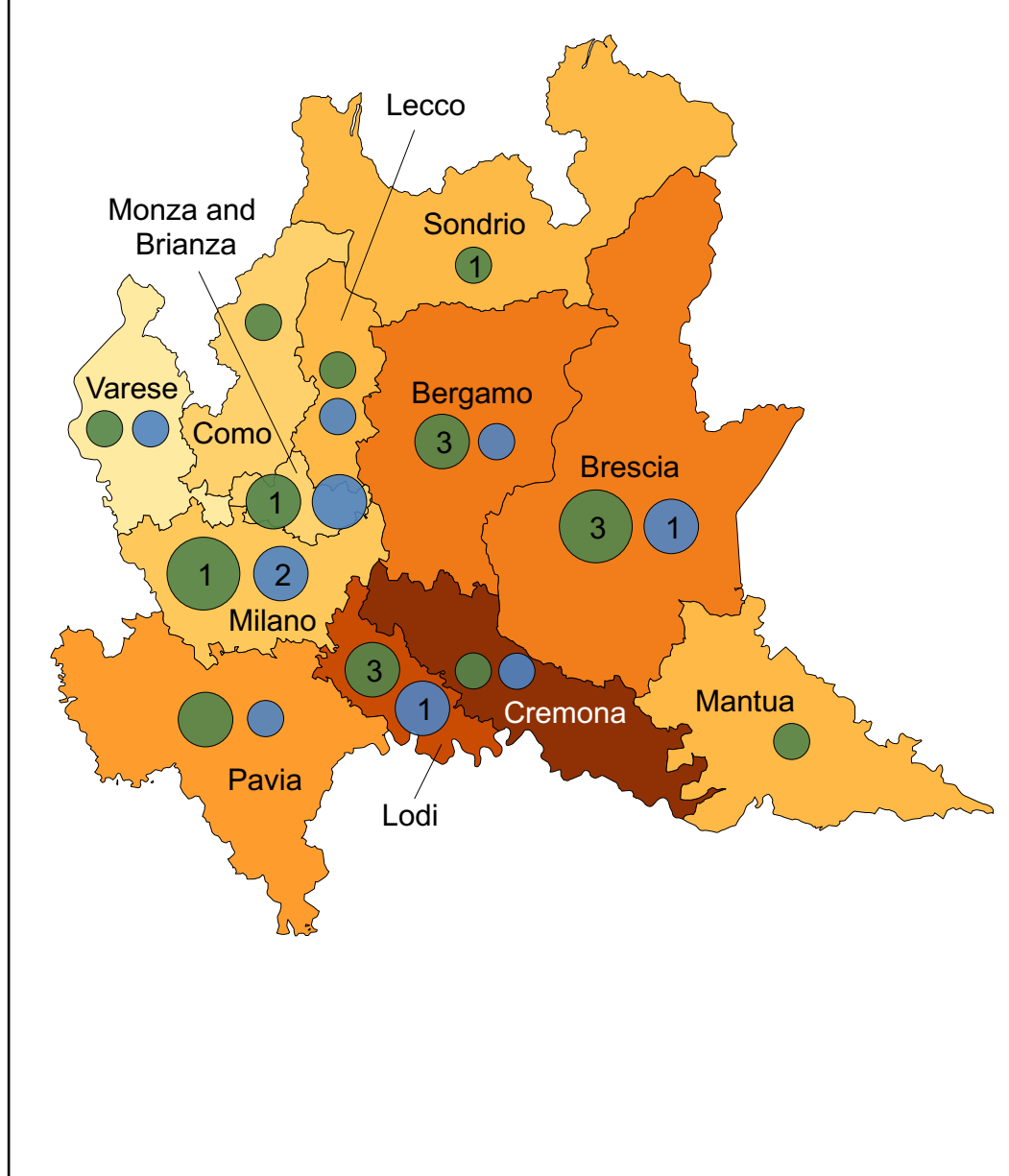
Number of Samples

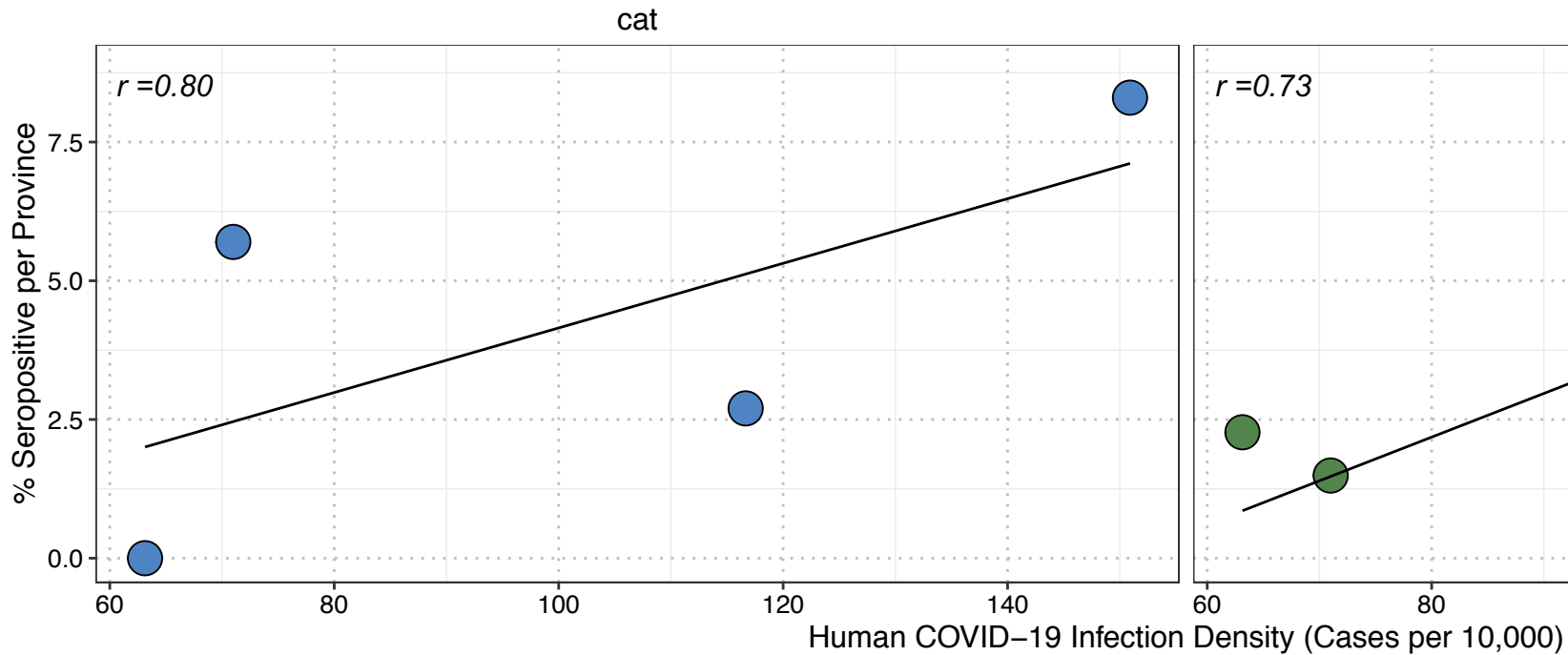


Italy



Region of Lombardy



A**B**