

SARS-CoV-2–Specific Antibodies in Domestic Cats during First COVID-19 Wave, Europe

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We conducted a severe acute respiratory syndrome coronavirus 2 antibody seroprevalence study among >2,000 domestic cats from 4 countries during the first coronavirus disease wave in Europe. We found 4.4% seroprevalence using a virus neutralization test and 4.3% using a receptor-binding domain ELISA, demonstrating probable human-to-cat transmission.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of the ongoing coronavirus disease (COVID-19) pandemic, causes high rates of illness and death among humans. SARS-CoV-2 is a newly recognized member of the genus *Betacoronavirus*, family *Coronaviridae*, that infects humans. An early serosurvey among domestic cats in Wuhan, China, during January–March 2020 reported 14.7% seropositivity (1). Experimental infections demonstrated susceptibility to SARS-CoV-2 infection in cats and other carnivore species, such as ferrets (*Mustela putorius furo*), minks (*Neovison vison*), and to a lesser extent domestic dogs (2,3), and confirmed anecdotal observations of naturally occurring human-to-animal transmissions (4,5). Respiratory and gastrointestinal signs were observed in SARS-CoV-2-infected cats (6–8). We conducted a seroprevalence study for SARS-CoV-2–specific antibodies among domestic cats in Europe during and after the first COVID-19 pandemic wave, using a plaque-reduction virus neutralization test (VNT) and a SARS-CoV-2 receptor-binding domain-specific ELISA (RBD-ELISA).

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The Study

We analyzed serum samples collected from 2,160 domestic cats during April–June 2020. Samples had been sent to a veterinary diagnostic laboratory (LABOKlin; Kissingen, Germany) for diagnostic purposes unrelated to suspicion of SARS-CoV-2 infection (9). Samples were from 1,136 cats in Germany, 331 in the United Kingdom, 333 in Italy, and 360 in Spain. Among 1,799 samples with demographic data, cats ranged from 0.1–23 years of age (median and mean age 11 years). We estimated a minimum of 300 total samples per location to enable a realistic estimation for each location. To confirm specificity of the assays to detect SARS-CoV-2–specific antibodies, we included 25 pre-pandemic cat serum samples and 25 serum samples from cats that tested positive for feline coronavirus/feline infectious peritonitis (FCoV/FIP) by NovaTec VetLine (Novatec Immundiagnostica GmbH, <https://www.novatec-id.com>), a commercial antibody test, in the screening.

We tested all serum samples by VNT, as previously described (10). We considered serum samples positive when titers were >20, expressed as the reciprocal of the dilution that gave >80% reduction of stained cells in the plaque reduction neutralization test (PRNT₈₀) (Appendix, <https://wwwnc.cdc.gov/EID/article/27/12/21-1252-App1.pdf>).

We also tested serum samples with an indirect ELISA we developed and validated inhouse. We used an ELISA previously used for detecting SARS-CoV-2 RBD antibodies in human serum (11) and replaced the anti-human IgG conjugate with an anti-cat IgG conjugate (Appendix).

We evaluated performance characteristics of the cat ELISA-RBD by using Pearson correlation of the results obtained by ELISA-RBD and Gaussian distribution analyses for the VNT. We also calculated

Table 1. Overall VNT SARS-CoV-2 seroprevalence in cats by country during the first pandemic wave, Europe, April–August 2020*

Location	No. tested	No. positive	% Positive (95% CI)†
Germany	1,136	48	4.2 (3.1–5.6)
United Kingdom	331	11	3.3 (1.7–5.9)
Italy	333	14	4.2 (2.3–7.0)
Spain	360	23	6.4 (4.1–9.4)
Total	2,160	96	4.4 (3.6–5.4)

*Seroprevalence determined by virus neutralization test (VNT). Similar results were found with RBD-ELISA, 4.3% (96/2,160; 95% CI 3.6%–5.4%) were seropositive (Table 2). RBD-ELISA, receptor-binding domain-specific ELISA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VNT, virus neutralization test.

†Calculated by using 2-sided exact binomial test in R (R Foundation for Statistical Computing, <https://www.r-project.org>).

diagnostic sensitivity and specificity of the ELISA-RBD compared with VNT. We conducted data analyses using R (R Foundation for Statistical Computing, <https://www.r-project.org>) and Prism version 9 (GraphPad Software Inc., <https://www.graphpad.com>). We calculated SARS-CoV-2 seroprevalence in cats separately for each country.

We found overall SARS-CoV-2 seroprevalence among cats was 4.2% in Germany, 3.3% in the United Kingdom, 4.2% in Italy, and 6.4% in Spain (Table 1; Figure). Among all 2,160 cat serum samples tested, 96 (4.4%, 95% CI 3.6%–5.4%) were positive by VNT and 92 (4.3%, 95% CI 3.4%–5.2%) by RBD-ELISA. The RBD-ELISA showed a diagnostic sensitivity of 90.6% (95% CI 90.0%–91.2%) and specificity of 99.8% (95% CI 99.8%–99.8%) compared with VNT (Table 2). Furthermore, correlation ($r = 0.9$, 95% CI 0.9–0.9) and Gaussian distribution analyses ($r^2 > 0.7$) revealed high agreement between VNT

and RBD-ELISA sensitivities. All 25 prepandemic serum samples and 25 FCoV/FIP-positive samples tested SARS-CoV-2–negative in both the VNT and RBD-ELISA (data not shown), confirming the specificity of the assay for measuring SARS-CoV-2-specific antibodies.

Our study of domestic cat serum from 4 selected countries showed that during the first COVID-19 wave in Europe, >4% of domestic cats had been infected with SARS-CoV-2, probably through their contacts with infected humans. Because serum samples were sent to the veterinary diagnostic laboratory for conditions unrelated to a suspected SARS-CoV-2 infection, our data might not fully represent the overall seropositivity of the domestic cat population in Europe.

We used a VNT and an RBD-ELISA based on the original SARS-CoV-2 wild-type isolate (Wuhan-Hu-1, GenBank accession no. MN908947.3). The RBD-ELISA proved to have a high sensitivity and specificity compared with the VNT (Table 2), but 5 low-titer (titer = 20) VNT-positive samples remained undetected by the RBD-ELISA. These samples might have remained undetected because of the high specificity of RBD-ELISA, which detects antibodies toward the single spike protein ectodomain. Unlike RBD-ELISA, VNT might identify a broader range of virus neutralizing antibodies, including those directed against other domains of the spike protein. Of note, the only correlation of virus protection we have to date is virus neutralization, which apparently correlates well with RBD-ELISA positivity. For serologic screening and for individual diagnostic testing of domestic cats, the RBD-ELISA could replace the VNT, thus avoiding

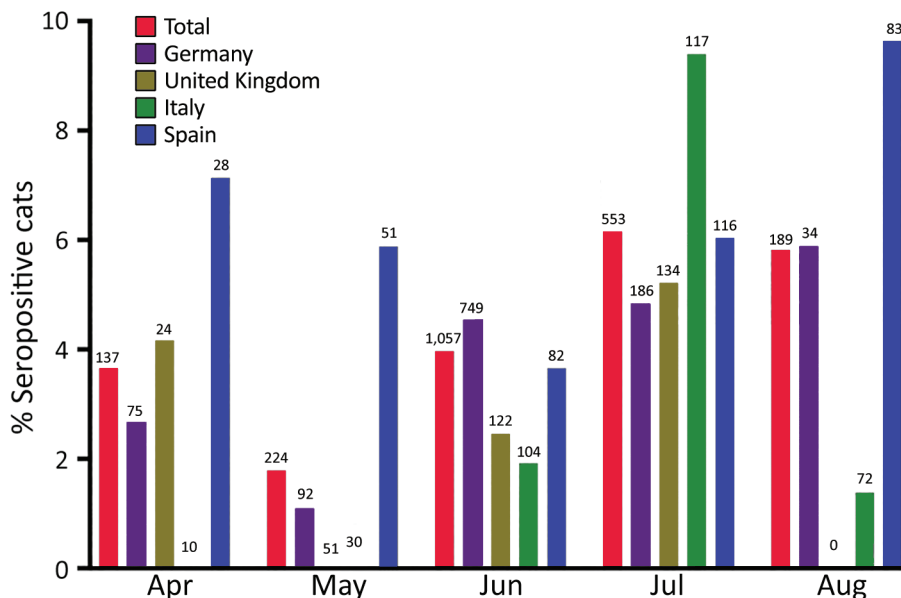


Figure. Overall seroprevalence of severe acute respiratory syndrome coronavirus 2 neutralizing antibodies in 2,160 domestic cats, by month and country, during the first coronavirus disease pandemic wave, Europe, April–August 2020. Numerals at the top of each column represent the number of samples collected. Seroprevalence rates peaked in July or August at ≤9.6% (95% CI 4.25%–18.11%) in Spain (Tables 1, 2).

the use of live SARS-CoV-2 under Biosafety Level 3 laboratory conditions. We further confirmed specificities of the VNT and RBD-ELISA by showing that pre-pandemic and FCoV/FIP-positive cat serum samples were negative in both assays. This finding excluded the detection of cross-reactive antibodies against feline alphacoronaviruses (4) and alphacoronaviruses of other animal species that might infect cats (4,12). Our data contrast a heavily affected area in China at the onset of the pandemic from which seropositivity levels of domestic cats ranged $\leq 15\%$ (1), although those results were from relatively fewer tested cats and used a different assay.

Conclusions

During the first COVID-19 pandemic wave, reported seroprevalence levels in domestic cats ranged from 0.4% in the Netherlands (4) to 23% among cats in COVID-19-positive households in France (13). Similar seroprevalence levels in cats and humans in the same areas found by us and others suggest that in the absence of another known source (4,13; C. Schulz, unpublished data) (Appendix Table), SARS-CoV-2 infections in cats are most likely due to human-to-cat contact transmission.

Most natural SARS-CoV-2 infections of cats appear to run a mild or subclinical course, with respiratory or gastrointestinal clinical signs reported in confirmed natural infections (6–8). Evidence from experimental studies suggests that cats are susceptible to SARS-CoV-2 infection and can maintain the virus within a cat population and spill the infection backward or forward to other species (2,3,14). However, no evidence of cat-to-human transmission, nor of cat-specific mutations or variants of SARS-CoV-2, has been detected thus far (8,12,15). This finding contrasts reports on minks kept in farms, where mink-to-human spillback infections and mink-specific mutations have been reported (5). Although no evidence currently suggests that domestic cats play a role in the epidemiology of human SARS-CoV-2 infection, clinicians and veterinary practitioners should recommend that SARS-CoV-2-infected persons avoid close contact with their domestic cats and practice the same nonpharmaceutical prevention measures toward cats as they do to prevent human-to-human infection.

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Table 2. Comparison of diagnostic sensitivity and specificity of the RBD-ELISA and VNT in a study of SARS-CoV-2 seroprevalence among domestic cats during the first pandemic wave, Europe, April–August 2020*

Test results	Value
RBD-ELISA sensitivity, % (95% CI)	90.6 (90.0–91.2)
RBD-ELISA specificity, % (95% CI)	99.8 (99.8–99.8)
No. positive (%; 95% CI), n = 2,160	
RBD-ELISA and VNT	87 (4.0; 3.2–4.9)
RBD-ELISA only	92 (4.3; 3.5–5.2)
VNT only	96 (4.4; 3.6–5.4)

*A total of 5 samples were positive with RBD-ELISA and negative with VNT; 9 samples were positive with VNT but negative with RBD-ELISA. RBD-ELISA, receptor-binding domain-specific ELISA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VNT, virus neutralization test.

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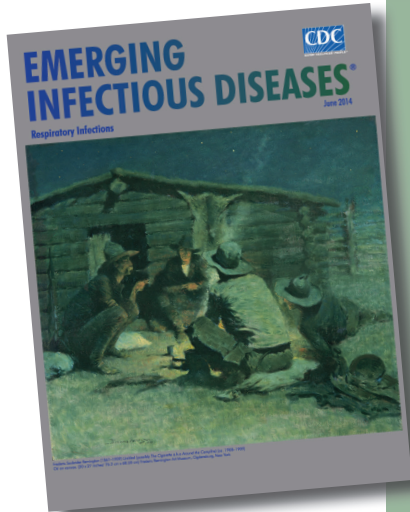
Zika [zēkə] Virus

Zika virus is a mosquito-borne positive-sense, single-stranded RNA virus in the family *Flaviviridae*, genus *Flavivirus* that causes a mild, acute febrile illness similar to dengue. In 1947, scientists researching yellow fever placed a rhesus macaque in a cage in the Zika Forest (*zika* meaning “overgrown” in the Luganda language), near the East African Virus Research Institute in Entebbe, Uganda. A fever developed in the monkey, and researchers isolated from its serum a transmissible agent that was first described as Zika virus in 1952. It was subsequently isolated from a human in Nigeria in 1954. From its discovery until 2007, confirmed cases of Zika virus infection from Africa and Southeast Asia were rare. In 2007, however, a major epidemic occurred in Yap Island, Micronesia. More recently, epidemics have occurred in Polynesia, Easter Island, the Cook Islands, and New Caledonia.

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Appendix

Virus Neutralization Test

We used a plaque reduction virus neutralization test (VNT) to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in cat serum samples as follows. We performed a 2-fold serial dilution of 1:20 diluted serum in culture medium and incubated with 500 50% tissue culture infectious dose (TCID₅₀) of SARS-CoV-2 strain Wuhan-Hu-1 for 1 h. Then we added the mixture to Vero cells and incubated for 1 h, after which we washed the cells and incubated in medium for another 8 h. We then fixed the cells with 4% paraformaldehyde and stained by using a rabbit anti–SARS-CoV serum (Sino Biological, <https://www.sinobiological.com>) and a secondary Alexa Fluor488–labeled goat anti-rabbit IgG (Invitrogen, <https://www.thermofisher.com>). We counted the number of infected cells per well by using the Cytation 1 imager (Biocompare, <https://www.biocompare.com>). Serum samples with titers >20, expressed as the reciprocal of the dilution that gave that gave >80% reduction of stained cells in the plaque reduction neutralization test (PRNT₈₀), were considered positive.

Receptor Binding Domain–Specific ELISA

We produced a SARS-CoV-2 receptor binding domain–specific ELISA (RBD-ELISA), as previously described (*1*). In brief, the RBD of spike 1 protein (S1) of SARS-CoV-2 residues belonging to strain SARS-CoV-2 Wuhan-Hu-1 were expressed through the expression plasmid pCAGGS in human embryonic kidney 293 cells (HEK-293T) cells. Constructs carried a C-terminal trimerization motif to induce RBD polymerization and a Strep-tag detection protein to facilitate RBD protein purification. In brief, we coated Costar plates (Corning Inc., <https://www.corning.com>) with 1 µg/mL of purified SARS-CoV-2 RBD antigen in 1%

phosphate-buffered saline (PBS) overnight at 4°C. Subsequently, we blocked plates with 1% skimmed milk powder (Campina, <https://www.campina.nl>) in 1% PBS and incubated for 1 h at 37°C. After blocking, we added 100 µL of 1:50 cat serum in blocking buffer containing 0.05% Tween-20, and 3% NaCl to each microplate well.

Controls on each plate included blank/conjugate only control, known positive cat serum controls, and archival serum negative control. We diluted secondary antibody, HRPO-labeled anti-cat (Rockland Immunochemicals, Inc., <https://rockland-inc.com>) at 1:10,000 in dilution buffer and then added the conjugate to each well and incubated for 1 h at 37°C. We filled each microwell with 100 µL of TMB-ELISA Substrate Solution (Thermo Fisher Scientific, <https://www.thermofisher.com>) for 10 min before stopping the reaction with same volume of 2 mol Alkaline Phosphatase Stop Solution (Sigma-Aldrich, <https://www.sigmaaldrich.com>). We read plates for optical density (OD) at 450 and 650 nm by using an RT-2100C Microplate Reader (Rayto Life and Analytical Sciences, <https://www.rayto.com>) within 30 min of stopping the reaction. We calculated the OD as the absorbance at 450 nm minus the OD at 650 nm to remove background noise before statistical analysis.

We set a change-point method according to available literature to establish the ELISA reliability and distinction between positive and negative cat serum samples for SARS-CoV-2 antibodies (16S). We determined optimal conditions to test the specificity of the RBD-ELISA. At a 1:50 serum dilution in RBD-ELISA, we observed an acceptable amount of background signal from the negative controls; at this dilution, the OD value of the positive control cat serum was ≥ 6 times the OD value of the negative control. Based on these data, we used a threshold of 3 SD above the mean of the negative controls for the RBD-ELISA. We considered serum samples with an OD value ≥ 0.1 nm positive for SARS-CoV-2 and those with an OD value < 0.1 nm negative. We calculated the diagnostic sensitivity and specificity of the RBD-ELISA compared with VNT, as described previously (2).

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Appendix Table. Seroprevalence in humans and cats by selected country during the first wave of the COVID-19 pandemic in Europe, 2020*

Country	Human		Cat (our study)		Reference	
	Seroprevalence	Month	Seroprevalence	Month	Human	Cat
Germany	0.9–2.2†	Mar–Jun†	0.7 (4.2)	Apr–Sep (Apr–Aug)	Fischer et al. 2020 (3)	Michelitsch et al. 2020 (4)
Italy, northern Italy	9 to 42%	Feb–Jul	3.9 (4.2)	Mar–May (Apr–Aug)	Signorelli et al. 2020 (5), Bassi et al. 2020 (6), Cito et al. 2020 (7)	Patterson et al. 2020 (8)
United Kingdom, Midlands	7%	Apr	– (4.2/5.8)	– (Apr/Apr–Aug)	Public Health England (9)	
Spain	5.0	Apr–May	–	–	Pollán et al. 2020 (10)	
Spain, Madrid	>10.0	Apr–May	– (6.3/6.4)	– (Apr–May/Apr–Aug)	Pollán et al. 2020 (10)	
France, COVID-19–positive households, Besancon	100	Jun	23.5 (–)	Jun (–)		Fritz et al. 2020 (11)
France, COVID-19 unknown status of households, Besancon	–	–	6.3 (–)	Jun (–)		Fritz et al. 2020 (11)
The Netherlands	2.7–9.5	April–May	0.4 (–)	April–May (–)	Slot et al. 2020 (12) (13), Vos et al. 2020	Zhao et al. 2021 (14)

*COVID-19, coronavirus disease; –, no data available.

†Low-incidence federal states of North-Rhine Westphalia, Lower Saxony, and Hesse.