

1 **SARS-CoV-2 and other coronaviruses in rats, Berlin, Germany, 2023**

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16 typing, phylogeny

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18 **Abstract**

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20 We tested 130 rats trapped in Berlin for coronaviruses. Antibodies against SARS-CoV-2 were
21 detected in a single animal only, but not in further 66 rats from the same location, speaking
22 against virus circulation in the rat population. All animals tested negative for SARS-CoV-2 by
23 RT-PCR. However, rodent-associated alphacoronaviruses were found.

24 **Main text**

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26 The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a betacoronavirus, was
27 initially reported in 2019 in China and thereafter spread rapidly worldwide, causing the
28 COVID-19 pandemic in humans. Since the pandemic unfolded, it was speculated about the
29 role of animals as amplifying or reservoir hosts. Because of the long-term association between
30 rodents and coronaviruses (1), the wide range of coronaviruses occurring in wild rodents (2)
31 and the ubiquitous distribution of commensal rodents, it was obvious to also include rodents
32 in susceptibility studies, among them rats. Under experimental conditions using high infection
33 doses, rats were reported as receptive particularly to the SARS-CoV-2 delta variant of
34 concern (VOC), but also experimental infection with other variants like alpha, beta or
35 omicron were described (3,4), posing the theoretical risk for establishing effective infection
36 chains in nature. Accordingly, field studies were initiated early into the pandemic to
37 investigate the situation in wild rats. Indeed, serological and molecular evidences of SARS-
38 CoV-2 infection of a few animals could be found in some studies (2,3,5), while others
39 reported consistently negative results (6,7). However, these studies were conducted before the
40 emergence and worldwide large-scale spread of the omicron VOC and its diverse subvariants.
41 In laboratory settings, lungs from omicron-infected animals showed significantly lower
42 infectious viral titers compared e.g. to delta (3), but field studies about omicron occurrence in
43 rat populations are missing. Therefore, we investigated rats trapped in Berlin, the very densely
44 populated (>4,000 inhabitants per km²) capital of Germany, during 2023, i.e. a period at
45 which omicron represented the dominant variant in the human population.

46 Lung and chest cavity lavage fluid samples were collected from 130 Norway or brown rats
47 (*Rattus norvegicus*) caught in the context of pest control at 44 trapping sites within Berlin
48 (Figure 1A). The lavage fluids were tested for antibodies against SARS-CoV-2 by a receptor
49 binding domain (RBD)-based multispecies ELISA using a cut-off of ≥ 0.3 for positivity as
50 described (8). Two orthologs of the RBD protein were used in parallel, the wild-type virus
51 RBD and that of the omicron XBB1.5 variant. The samples were prediluted 1/10 as described
52 for lavage samples of rodents (6). One of the 130 rats tested positive, the optical density (OD)
53 values were 1.16 (wild-type RBD) and 1.53 (omicron RBD), respectively. To confirm the
54 positive results, the sample was additionally tested by a surrogate virus neutralization test
55 (sVNT) (cPass SARS-CoV-2 Neutralization Antibody Detection Kit, GenScript, the
56 Netherlands) performed as prescribed by the manufacturer (cut-off for positivity at $\geq 30\%$)

57 inhibition). In its original composition, the test enables the detection of antibodies against the
58 wild-type virus and all VOCs except omicron. For omicron and its sub-variants, a specific
59 RBD is provided by the test manufacturer. The rat sample positive in the RBD-ELISA was
60 analyzed by the sVNT using the original and the omicron-specific RBD, and the omicron-
61 based test gave a positive result (33.9% inhibition; 23.4% for the wild-type RBD). These
62 results hint at a previous infection of the animal with an omicron subvariant. However, that
63 only one rat tested positive speaks very clearly for a single spillover event from the human
64 into the rat population and against autonomous virus circulation in rats, especially as further
65 66 rats were caught in the same building as the sero-reactive animal and all of them tested
66 negative (Figure 1A).

67 To further confirm that there is no ongoing virus circulation in the sampled rat population, we
68 tested the lungs by a SARS-CoV-2-specific real-time RT-PCR targeting the RNA-dependent
69 RNA polymerase (RdRp) gene (9) and by a likewise RdRp-based, generic pan-coronavirus
70 RT-PCR (10). In the SARS-CoV-2-specific test, all samples scored negative, verifying the
71 absence of SARS-CoV-2 in the analyzed samples from Berlin. Nevertheless, five lung
72 samples were positive in the pan-coronavirus RT-PCR; all five animals were trapped at the
73 same location (Figure 1). For further characterization, the RT-PCR products were sequenced
74 in both directions with the primers used for amplification. The amplicon sequences (NCBI
75 GenBank accession numbers OR854629-OR854633) were subsequently compared to
76 representative coronavirus sequences obtained from GenBank. Virus typing based on the
77 partial RdRp sequences revealed that the viruses found in Berlin rats belong to the genus
78 *Alphacoronavirus* and are closely related to each other (99.4-100.0% identity on nucleotide
79 level) and to the Lucheng Rn rat coronavirus (Figure 1B). Hence, in contrast to SARS-CoV-2,
80 rodent-associated alphacoronaviruses appear to circulate in the investigated rat population,
81 which is in line with previous studies investigating coronaviruses in rats (2,5).

82 Viral monitoring of rodent populations like rats is essential to understand e.g. virus
83 occurrence, transmission characteristics and pathogenesis, not only for their potential impact
84 on rodents but also due to the potential for recombination and the zoonotic nature of
85 coronaviruses. Research into rodent coronaviruses contributes to a broader understanding of
86 these viruses and aids in the development of strategies for managing both animal and public
87 health.

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100 **Ethical Statement**

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102 The rat samples were collected in the context of rodent pest control, which did not require a
103 specific permit.

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105 **References**

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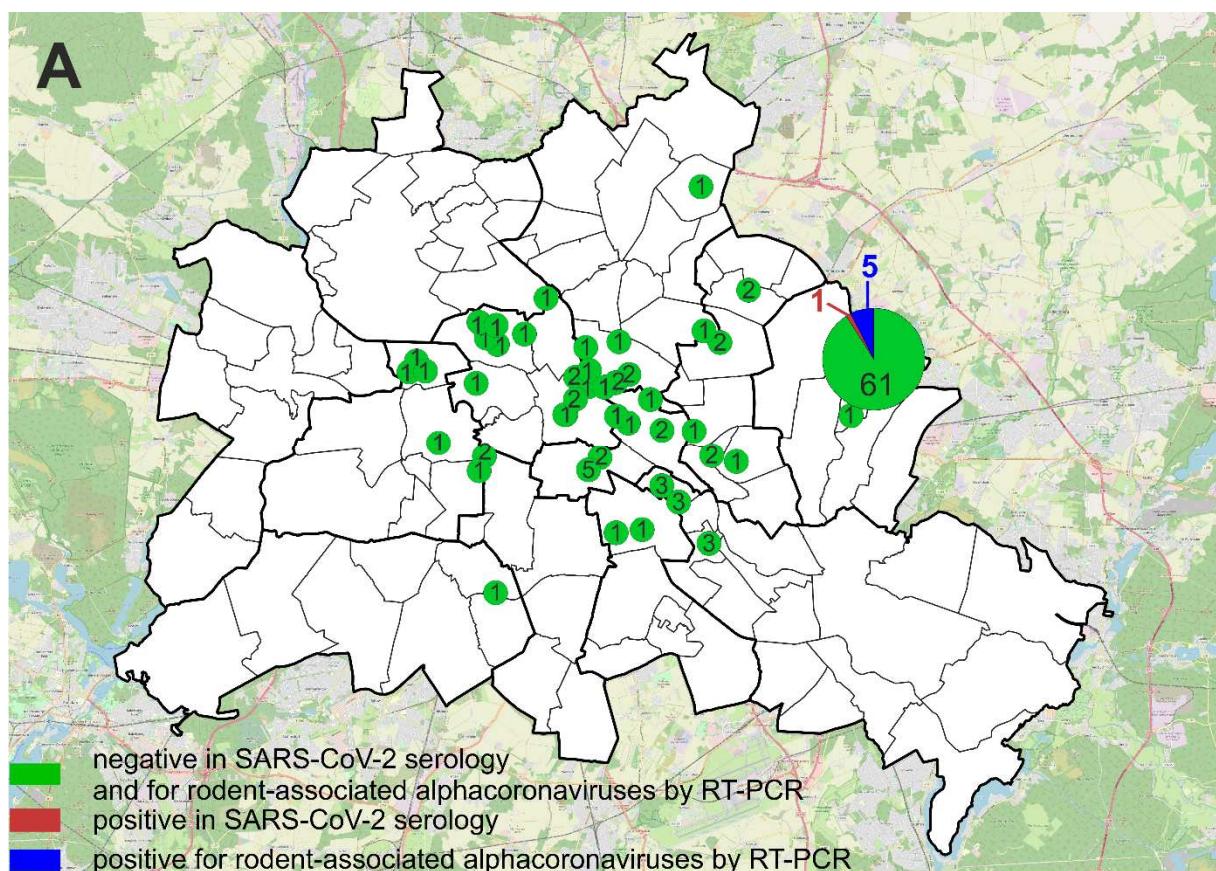
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135 Zimbabwe. Viruses. 2022 Apr 9;14(4).

136 **Figure Legend**

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138 **Figure 1. A.** Locations within Berlin at which rats were trapped and number of animals per
139 location. Green dots represent areas from which all sampled animals tested negative in the
140 SARS-CoV-2 RBD-based ELISA and negative for coronaviruses by RT-PCR. When rats
141 tested positive for coronaviruses by RT-PCR, the number of animals is given in blue. The
142 single animal that tested positive for antibodies against SARS-CoV-2 is indicated in red. The
143 map of Berlin, in which the dots were printed, was retrieved from Geoportal Berlin, dataset
144 "Geoportal Berlin / Ortsteile von Berlin", URL: <https://daten.odis-berlin.de/de/dataset/ortsteile/>, data license Germany – attribution – Version 2.0
145 (www.govdata.de/dl-de/by-2-0). The map of the area surrounding Berlin was retrieved from
146 OpenStreetMap (map data copyrighted OpenStreetMap contributors and available from
147 <https://www.openstreetmap.org>). **B.** Classification of the detected coronaviruses based on
148 partial sequences of the RNA-dependent RNA polymerase gene. The Maximum-likelihood
149 tree was calculated by using the MEGA X software. Statistical support for nodes was obtained
150 by bootstrapping (1,000 replicates); only values $\geq 50\%$ are shown. Virus names are preceded
151 by the respective NCBI GenBank accession number. Sequences generated during this study
152 are marked in red. The chart background of viruses belonging to the same coronavirus genus
153 is highlighted by the same color and the genera are indicated.
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