SARS-CoV-2 and Other Coronaviruses in Rats, Berlin, Germany, 2023

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DOI: https://doi.org/10.3201/eid3010.241079

We tested 130 rats captured in Berlin for coronaviruses. SARS-CoV-2 antibodies were detected in 1 rat, but all animals were negative by reverse transcription PCR, suggesting SARS-CoV-2 was not circulating in the rat population. However, alphacoronaviruses were found. Monitoring rodent populations helps to determine coronavirus occurrence, transmission, and zoonotic potential.

SARS-CoV-2 was initially reported in 2019 in China and spread rapidly worldwide, causing the COVID-19 pandemic in humans. Since the pandemic unfolded, the role of animals as amplifying or reservoir hosts has been hypothesized. Because of the long-term association between rodents and coronaviruses (*1*), the wide range of coronaviruses occurring in wild rodents (*2*), and the ubiquitous distribution of commensal rodents, investigations of SARS-CoV-2 and other coronaviruses in rats is warranted. In experiments that used high infection doses, rats have been reported as receptive SARS-CoV-2 hosts, particularly for the Delta variant of concern (VOC); however, experimental infections with Alpha, Beta, and Omicron variants have also been described in rats (*3*,*4*), suggesting a theoretical risk for effective transmission chains in nature. Accordingly, field studies were initiated early during the pandemic to investigate SARS-CoV-2 infections in wild rats. Indeed, serologic and molecular evidence of SARS-CoV-2 infection was found in a few animals in some studies (*2*,*3*,*5*), whereas other studies consistently reported negative results (*6*,*7*). However, all of those studies were conducted before the emergence and worldwide large-scale spread of the Omicron VOC and its subvariants. In laboratory settings, lungs from Omicron virus–infected rats showed significantly lower infectious virus titers compared with rats infected with the Delta variant (*3*), but field studies on wild rats after Omicron VOC emergence and dominance within the human populations are missing. Therefore, we investigated SARS-CoV-2 infections in Norway rats (*Rattus norvegicus*) captured in Berlin, the very densely populated (>4,000 inhabitants/km^2) capital of Germany, during 2023, when Omicron was the dominant SARS-CoV-2 variant in

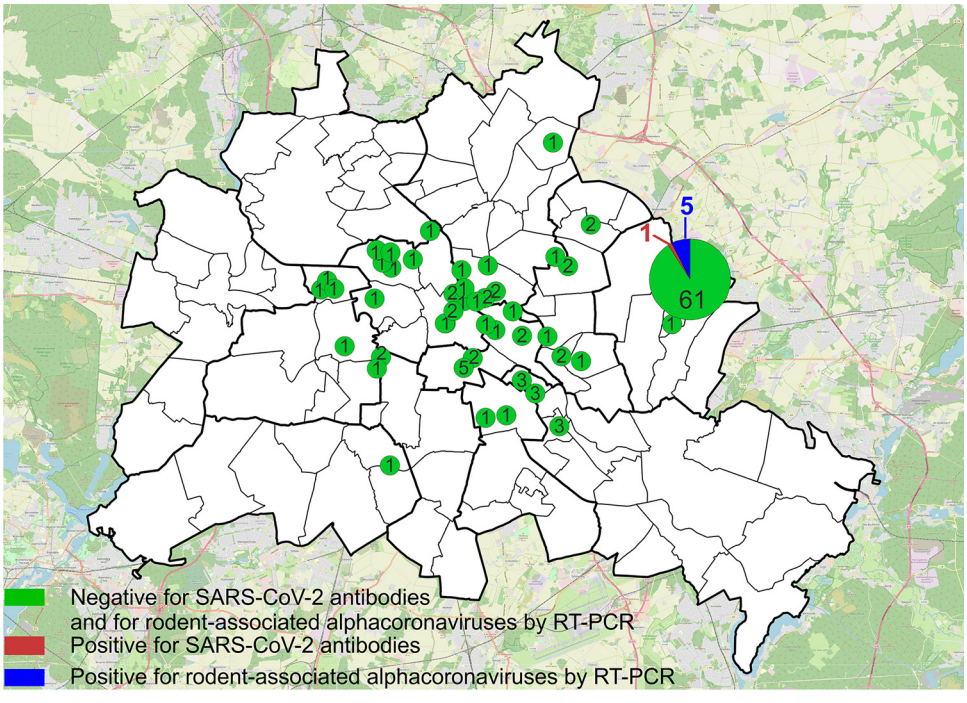

Figure 1. Locations of trapped rats tested in study of SARS-CoV-2 and other coronaviruses in rats, Berlin, Germany, 2023. Numbers indicate numbers of rats tested in each location. Overlay map of Berlin, in which the circles were printed, was retrieved from Geoportal Berlin, dataset Geoportal Berlin/ Ortsteile von Berlin (https://daten.odis-berlin.de/de/dataset/ortsteile), data license Germany–attribution–Version 2.0 (https://www.govdata.de/dl-de/by-2-0). Map of the area surrounding Berlin was created by using OpenStreetMap (https://www.openstreetmap.org). RT-PCR, reverse transcription PCR.

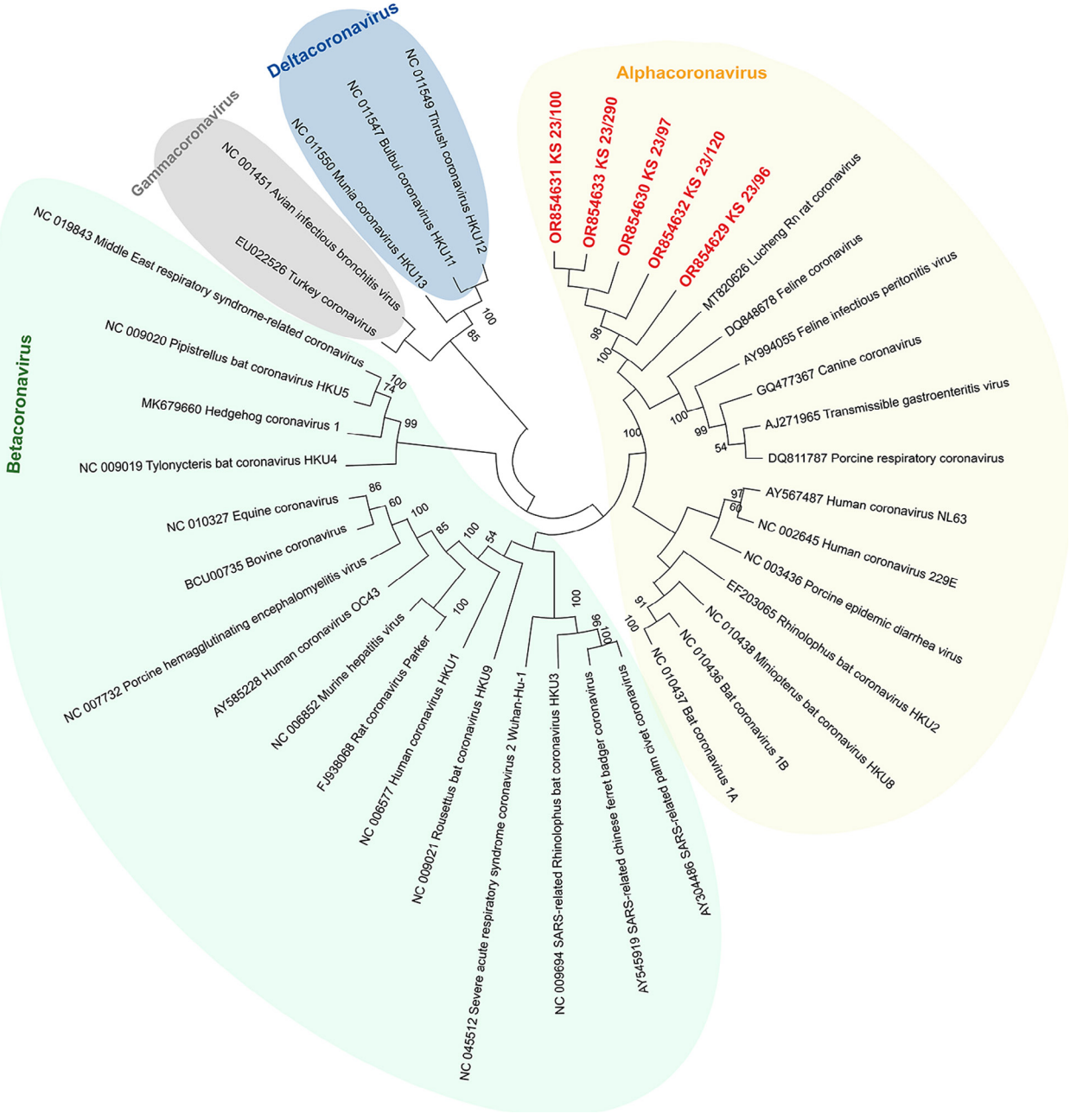

Figure 2. Phylogenetic analysis of SARS-CoV-2 and other coronaviruses in rats, Berlin, Germany, 2023. Partial sequences of the RNA-dependent RNA polymerase gene from coronaviruses isolated from rats in Berlin (red text) were compared with other coronavirus sequences obtained from GenBank. Background colors indicate viruses belonging to the same coronavirus genus. The maximum-likelihood tree was calculated by using MEGA X software (https://www.megasoftware.net). Statistical support for nodes was obtained by bootstrapping (1,000 replicates); only bootstrap values ≥50% are shown. GenBank accession numbers are provided. Tree not drawn to scale.

the human population. Rat samples were collected during rodent pest control activities; sample collection did not require a specific permit.

We collected samples of lung and chest cavity lavage fluid from 130 Norway rats caught at 44 trapping sites (Figure 1) by rinsing the chest cavity with 1 mL phosphate-buffered saline during necropsy. We tested lavage fluids for antibodies against SARS-CoV-2 by using a receptor-binding domain (RBD)–based multispecies ELISA and a cutoff value of ≥0.3 for positivity, as previously described (*8*). We used 2 RBD protein orthologs in parallel, the wild-type

virus RBD and that of the Omicron XBB1.5 variant. We prediluted the samples 1:10 as described for rodent lavage samples (*6*).

One of 130 rats tested positive; the optical density values were 1.16 (wild-type RBD) and 1.53 (Omicron RBD) (Appendix Figure, https://wwwnc.cdc.gov/EID/article/30/10/24-1079-App1.pdf). To confirm the positive result, we tested the sample by using a surrogate virus neutralization test (cPass SARS-CoV-2 Neutralization Antibody Detection Kit; GenScript, https://www.genscript.com) and 2 different RBD orthologs according to the manufacturer's instructions (cutoff for positivity was ≥30% inhibition). That test, in its original composition, enables the detection of antibodies against wild-type SARS-CoV-2 and all VOCs except Omicron. For Omicron and its subvariants, we used a specific RBD provided by the manufacturer (GeneScript). The ELISA-positive rat sample was positive against Omicron-specific RBD in the neutralization test (33.9% inhibition for Omicron, 23.4% for wild-type RBD), suggesting the rat had a previous infection with an Omicron subvariant. However, only 1 rat tested positive, indicating a single spillover event from humans into the rat population and lack of autonomous virus circulation in rats, especially considering 66 additional rats were caught in the same building as the seroreactive animal (Figure 1), and all of those tested negative. Potential cross-reactivity with other coronaviruses could account for the single positive result, although cross-reactivity of some animal coronaviruses was excluded during initial validation of the RBD-based ELISA (*8*).

To further confirm that no virus circulated in the sampled rat population, we tested lung samples by using SARS-CoV-2–specific real-time reverse transcription PCR (RT-PCR) targeting the RNA-dependent RNA polymerase (*RdRp*) gene (*9*) and by using an *RdRp*-based, generic pancoronavirus RT-PCR (*10*). Using the SARS-CoV-2–specific test, all samples were negative, verifying the absence of SARS-CoV-2. Nevertheless, 5 lung samples were positive in the pancoronavirus RT-PCR; all 5 animals were trapped at the same location (Figure 1). For further characterization, we sequenced the RT-PCR products in both directions by using the PCR amplification primers. We deposited the sequences in GenBank (accession nos. OR854629–33) and compared them with other representative coronavirus sequences obtained from GenBank. Virus typing according to the partial *RdRp* sequences revealed that the viruses found in Berlin rats belonged to the genus *Alphacoronavirus* and were closely related to each other (99.4%–100.0% nucleotide sequence identity) and to the Lucheng Rn rat coronavirus (Figure 2). Hence, in contrast to SARS-CoV-2, rodent-associated alphacoronaviruses were circulating within the Berlin rat population, which agrees with previous studies of coronaviruses in rats in other locations (*2*,*5*).

In conclusion, research into rodent coronaviruses contributes to a broader understanding of those viruses and aids in the development of strategies for managing both animal and public health. Coronavirus monitoring of rodent populations aids in determining virus occurrence, transmission characteristics, pathogenesis, and zoonotic potential.

Acknowledgments

We thank Bianka Hillmann, Dennis Karnatz, and Janina Beyer for excellent technical assistance, our institute colleagues for help during animal dissections, and the rodent pest controllers from Berlin for providing rats.

The study was supported by intramural funding from the German Federal Ministry of Food and Agriculture provided to the Friedrich-Loeffler-Institut, partial funding to M.B. from the European Union Horizon 2020 project (Versatile Emerging Infectious Disease Observatory, grant no. 874735), and funding to R.G.U. from the German Center for Infection Research, thematic translational unit Emerging Infections.

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Establishment of *Amblyomma maculatum* Ticks and *Rickettsia parkeri* in the Northeastern United States

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DOI: https://doi.org/10.3201/eid3010.240821

We document a case of *Rickettsia parkeri* rickettsiosis in a patient in Connecticut, USA, who became ill after a bite from a Gulf Coast tick (*Amblyomma maculatum*). We used PCR to amplify *R. parkeri* DNA from the detached tick. The patient showed a 4-fold rise in IgG reactive with *R. parkeri* antigens.

Native and invasive tick species pose serious public health concerns in the United States, particularly in northeastern states. Recent and rapid expansion of the lone star tick (*Amblyomma americanum*) into ranges with pervasive blacklegged tick (*Ixodes scapularis*) populations has increased the number of recognized tickborne pathogens that circulate in that densely populated region. In addition to *Borrelia burgdorferi*, the causative agent of Lyme disease, ≥7 additional tickborne pathogens are now endemic to the northeastern United States: *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, Heartland virus, *Anaplasma phagocytophilum*, *Borrelia miyamotoi, Babesia microti*, and Powassan virus (*1*). Multiple factors, including climate change and anthropogenic modifications to the environment, have affected rapid expansion of the ranges of medically relevant tick species and associated pathogens. That expansion has been reflected by dramatic increases in the numbers of reported cases of tickborne diseases in the northeastern United States since the beginning of the 21st Century (*1*).

The Gulf Coast tick (*Amblyomma maculatum*) was first identified in the United States in 1844. As recently as the middle of the 20th Century, the tick's range was restricted predominantly to coastal regions of states bordering the Gulf of Mexico as far west as Texas and the southern Atlantic coast only as far north as southern North Carolina (Figure 1) (*2*,*3*). Established *A. maculatum* tick populations now exist in states hundreds of miles inland (Arkansas, Missouri, Kentucky, Illinois, Indiana) and along the Atlantic coast as far north as Connecticut (*4*–*9*). Migratory grassland birds serve a crucial role in the spread of Gulf Coast ticks to locations in central and northern states that possess favorable environmental conditions for the tick's survival (*2*,*8*).

The Gulf Coast tick was relatively unknown and infrequently studied until recognition of *Rickettsia parkeri* spotted fever rickettsiosis in 2004 (*2*). In contrast to its more widely recognized cousins, blacklegged and lone star ticks, which prefer predominantly woodland habitats, Gulf Coast ticks favor grassland habitats. During the past 250 years, huge swathes of native grasslands and savannahs in the eastern United States have been transformed into agricultural areas and rangeland, creating habitats no longer favorable for Gulf Coast ticks. Paradoxically, contemporary reclamation of native grasslands through conservation