

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Pangolin v3.1.11, 2021-09-17 was used to assign lineages to SARS-CoV-2 sequences.

Data analysis

IQ-TREE version v1.6.12 (open source) was used to infer phylogenetic trees which were visualized in FigTree v.1.4.4 (open source). TempEst v1.5.3 (open source) was used to assess incongruence between genetic divergence and sampling date. BEAST v1.10.4 package (open source) was used to perform the time-scaled Bayesian analysis using the Markov chain Monte Carlo method and results were visualized in Tracer v.1.7.1 (open source). LogCombiner v1.10.4 (open source) was used to combine runs/trees which was summarized into a single tree by by TreeAnnotator v1.10.4 (open source). The National Veterinary Services Laboratory vSNP pipeline was applied for SNP analysis and used Wuhan-Hu-1 (NC_045512) as a reference. The pipeline is available here: <https://github.com/USDA-VS/vSNP>.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Whole-genome sequences are available on GISAID and SRA for all 14 deer viruses sequenced for this study, accession numbers are available in Extended Data Table 4. All other data analyzed in this paper is available as part of the Article, Extended Data Tables 1-4. All SARS-CoV-2 sequences from humans in Ohio (Jan 1, 2020-March 31, 2021) were compiled from GISAID and downloaded 10/8/2021. B.1.596 sequences were sequences were pulled from NCBI's GenBank. Number of SARS-CoV-2 cases in Ohio from April 2020-September 2020 was pulled from the US Centers for Disease Control and Prevention COVID-19 Surveillance website: <https://data.cdc.gov/Case-Surveillance/COVID-19-Case-Surveillance-Public-Use-Data-with-Ge/n8mc-b4w4>

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	This study involved SARS-CoV-2 testing in white-tailed deer followed by epidemiological analyses of positive samples, and phylogenetic analyses of SARS-CoV-2 sequences.
Research sample	Deer were harvested as part of a deer population management program in northeast Ohio and not for the purposes of this study. Sample collection was opportunistic and conducted in cooperation with regulatory agencies and as part of routine surveillance programs. Harvested animals ranged in age from from ~0.5 to 5.5 years old and included 149 males and 215 females which generally represented the broader deer population around northeast Ohio. There were limitations on the number of antlered deer that could be harvested which contributed to the lower number of males included in this study. Deer harvest occurred at locations that were baited for up to two weeks prior to each culling session, and additional deer were harvested opportunistically when they were observed away from the bait on a culling session day.
Sampling strategy	We sampled all deer that were harvested between at our study sites Jan-Mar 2021 as part of the northeast Ohio deer population management program were sampled as part of this study.
Data collection	Patricia Dennis and Jennifer Grieser were responsible for conducting or overseeing sample collection. This included recording the age, weight, sex, and reproductive status of each deer using pen and paper, and they collected a nasal swab into BHIB. Samples were then shipped to Andrew Bowman where Jacqueline Nolting, Christopher Madden, and Devra Huey extracted and qPCR tested, and recorded assay results from each sample in Excel spreadsheets. Positive samples then underwent sequencing. Mary Killian, Kristina Lantz, Rachel Tell, Mia Torchetti, Suelee Robbe-Austerman, Martha Nelson, Dillon McBride and Seth Faith were involved in SARS-CoV-2 sequence analysis, phylogenetic analyses, and download of publicly available SARS-CoV-2 sequences.
Timing and spatial scale	All samples were collected between January-March 2021 from 9 different sites in northeast Ohio spread across a 1000 sq km area.
Data exclusions	The only data excluded from analysis were SARS-CoV-2 sequences identified as "outlier sequences" by TempEst due to long branch lengths and incongruence between genetic divergence and sampling date. This can occur due to poor sequence coverage. One of the 14 sequences obtained from deer in our study (hCoV-19/deer/USA/OH-OSU-0025/2021, site 4) was lower in coverage and had a very long branch length and was excluded from the final phylogenetic analysis.
Reproducibility	All deer nasal swabs were initially tested using the Charité/Berlin (WHO) assay. Briefly, this included an E assay. If the E assay was positive, it was followed by an RdRp confirmatory and discriminatory assay. All E assay positive samples were also retested with the CDC, and samples that were CDC positive were classified as presumptive positives. NVSL confirmed 75 of 76 attempted.
Randomization	As SARS-CoV-2 infection status was unknown in all deer prior to testing, all samples were extracted in batches without any specific efforts toward randomization.
Blinding	SARS-CoV-2 infection status was unknown in all deer prior to testing.
Did the study involve field work?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

Field work, collection and transport

Field conditions	Samples were collected from northeast Ohio (USA) in January-March 2021 (average temperature range during these months is 20.3F-51.7F).
Location	All deer came from northeast Ohio (USA) and were harvested and transported to a central processing point where nasal swabs were

Location	collected from each deer. Deer were harvested as part of a deer population management program and not for the purposes of this study.
Access & import/export	Deer were harvested as part of a deer population management program in northeast Ohio and not for the purposes of this study. Sample collection was opportunistic and conducted in cooperation with regulatory agencies and as part of routine surveillance programs. Samples were collected post-mortem, which was deemed exempt by the Ohio State University Institutional Animal Care and Use Committee.
Disturbance	N/A

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	No laboratory animals were used.
Wild animals	Male and female, 0.5 - 5.5 year-old deer (<i>Odocoileus virginianus</i>) were harvested as part of a deer population management program in northeast Ohio and not for the purposes of this study. Sample collection was opportunistic and occurred posthumously.
Field-collected samples	Harvested deer carcasses were transported to a central processing point where samples were collected. Sample collectors wore gloves and a facemask. A nasal swab was collected from each deer and placed into a tube with brain heart infusion broth (BHIB). After collection, samples were immediately chilled on ice packs then transferred into a -80°C freezer within 12 h where they remained until testing was initiated.
Ethics oversight	The animal-origin samples tested in the manuscript were collected after the animals were harvested for purposes not related to this research; therefore, this study was deemed exempt by The Ohio State University's Institutional Animal Care and Use Committee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.