SARS-CoV-2 infection in free-ranging white-tailed deer

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Humans have infected a wide range of animals with SARS-CoV-2[1–5], but the establishment of a new natural animal reservoir has not been observed. Here we document that free-ranging white-tailed deer (*Odocoileus virginianus*) are highly susceptible to infection with SARS-CoV-2, are exposed to multiple SARS-CoV-2 variants from humans and are capable of sustaining transmission in nature. Using real-time PCR with reverse transcription, we detected SARS-CoV-2 in more than one-third (129 out of 360, 35.8%) of nasal swabs obtained from *O. virginianus* in northeast Ohio in the USA during January to March 2021. Deer in six locations were infected with three SARS-CoV-2 lineages (B.1.2, B.1.582 and B.1.596). The B.1.2 viruses, dominant in humans in Ohio at the time, infected deer in four locations. We detected probable deer-to-deer transmission of B.1.2, B.1.582 and B.1.596 viruses, enabling the virus to acquire amino acid substitutions in the spike protein (including the receptor-binding domain) and ORF1 that are observed infrequently in humans. No spillback to humans was observed, but these findings demonstrate that SARS-CoV-2 viruses have been transmitted in wildlife in the USA, potentially opening new pathways for evolution. There is an urgent need to establish comprehensive 'One Health' programmes to monitor the environment, deer and other wildlife hosts globally.

As of 9 November 2021, SARS-CoV-2, the virus responsible for coronavirus disease 2019 (COVID-19), has caused more than 5 million deaths globally[6]. The zoonotic origins of SARS-CoV-2 are not fully resolved[7], exposing large gaps in our knowledge of susceptible host species and potential new reservoirs. Natural infections of SARS-CoV-2 linked to human exposure have been reported in domestic animals such as cats, dogs and ferrets, and in wildlife under human care, including several species of big cats, Asian small-clawed otters, western lowland gorillas and mink[1]. Detection of SARS-CoV-2 by PCR in free-ranging wildlife has been limited to small numbers of mink in Spain and in Utah in the USA, which were thought to have escaped from nearby farms[8,9]. An in silico study modelling SARS-CoV-2 binding sites on the angiotensin-converting enzyme 2 (ACE2) receptor across host species predicted that cetaceans, rodents, primates and several species of deer are at high risk of infection[10]. Experimental infections have identified additional animal species susceptible to SARS-CoV-2, including hamsters, North American raccoons, striped skunks, white-tailed deer, raccoon dogs, fruit bats, deer mice, domestic European rabbits, bushy-tailed woodrats, tree shrews and multiple non-human primate species[11–20]. Moreover, several species are capable of intraspecies SARS-CoV-2 transmission[13–15,17,21–23], including cats, ferrets, fruit bats, hamsters, raccoon dogs, deer mice and white-tailed deer. Vertical transmission has also been documented in experimentally infected white-tailed deer[23]. In July 2021, antibodies for SARS-CoV-2 were reported in 152 free-ranging white-tailed deer (seroprevalence 40%) sampled across Michigan, Pennsylvania, Illinois and New York in the USA[24], raising the possibility that SARS-CoV-2 has infected deer in the Midwest and northeast regions.

In this study, we report the detection of SARS-CoV-2 in 129 out of 360 (35.8%) free-ranging white tailed deer (*O. virginianus*) from northeast Ohio using real-time PCR with reverse transcription (rRT–PCR) between January and March 2021. SARS-CoV-2 is a reportable disease in animals, and per international health regulations, these results were reported immediately to the World Organisation for Animal Health (OIE) on 31 August 2021 (Report ID: FUR_151387, Outbreak ID: 89973)[25]; this was the first PCR-confirmed report of natural infection of SARS-CoV-2 in a cervid globally. Whole-genome sequences of 14 SARS-CoV-2 viruses were deposited in GISAID on 5 October 2021 (Extended Data Table 4). Additionally, we recovered two viable SARS-CoV-2 isolates from our

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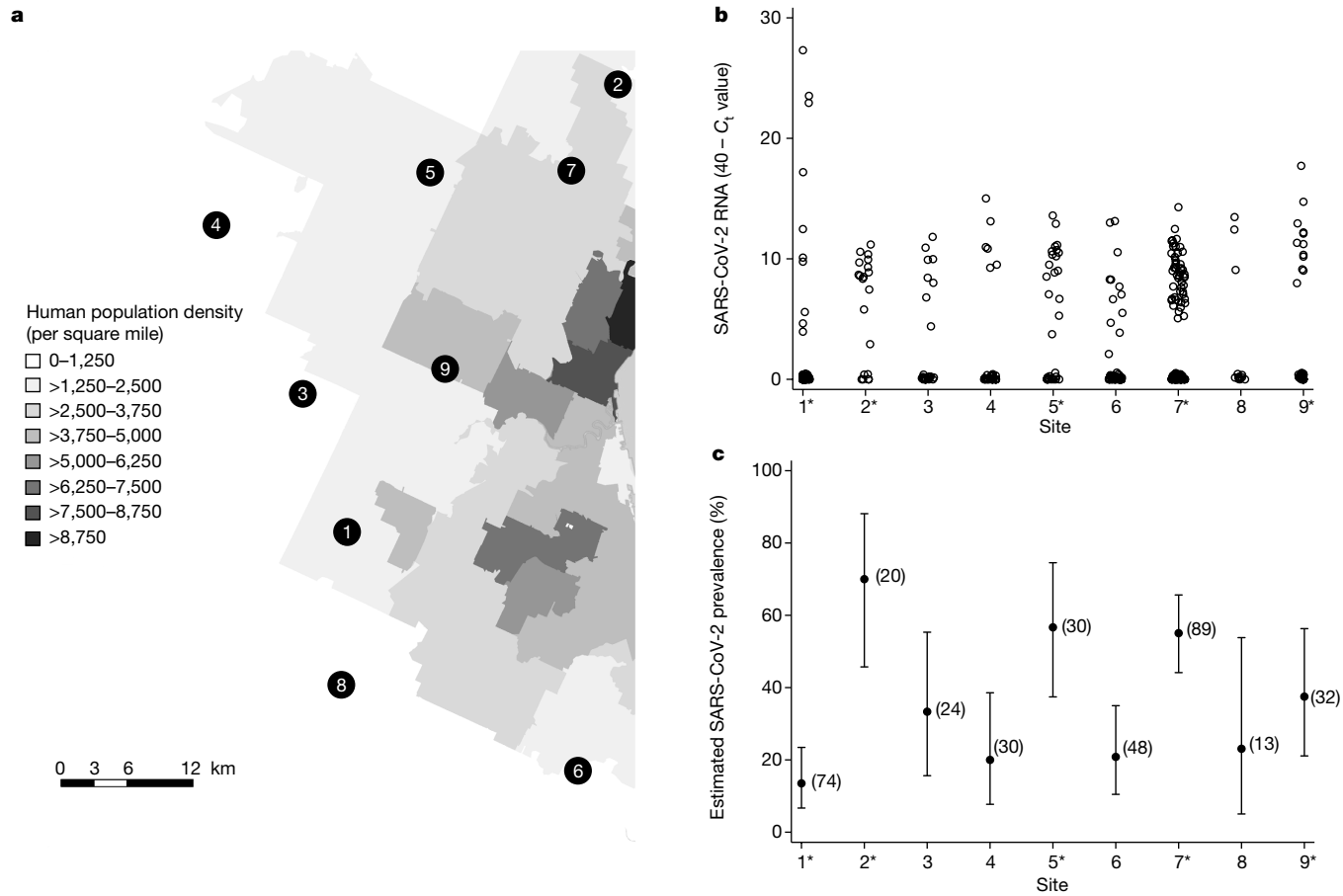

Fig. 1 | SARS-CoV-2 viral RNA in white-tailed deer across the study locations. a, The nine study sites were spread across a 1,000-km^2 landscape of varying population density in northeast Ohio. Darker shading corresponds to higher human population density (people per square mile). Sampling sites 1, 2, 5, 7 and 9 are in close proximity to human populations and are indicated as urban sites with an asterisk in **b**, **c**. **b**, Nasal swabs from white-tailed deer were tested for the presence of SARS-CoV-2 viral RNA using real-time reverse transcriptase PCR (rRT–PCR). Estimates of amount of SARS-CoV-2 viral RNA are represented by (40 – C_t value of the N1 rRT–PCR target). Negative samples are represented with a value of zero. **c**, The prevalence of SARS-CoV-2 in the white-tailed deer at each study site was estimated using rRT–PCR. The proportion of positive samples is shown with Clopper–Pearson exact 95% confidence interval bars. The number of samples collected at each site is indicated in parentheses. Map created with ArcMap v.10.8.1, using base layers and data from Esri, Garmin, OpenStreetMap, GIS user community, Infogroup and the US Census Bureau.

samples, providing evidence that naturally infected deer shed infectious SARS-CoV-2 virus. We used genetic sequence data to estimate the number of human-to-deer transmission events, characterize the genetic diversity of the virus in deer and identify phylogenetic clades of deer-only viruses arising from deer-to-deer transmission.

High infection rate of SARS-CoV-2

We sampled 360 free-ranging white-tailed deer across from 9 locations (Fig. 1a) in northeast Ohio between January and March 2021. Across all sites, SARS-CoV-2 was detected by rRT–PCR in 35.8% of nasal swabs from white-tailed deer (129 out of 360, 95% confidence interval 30.9–41.0%) (Supplementary Table 1). Each site was sampled 1–3 times during the study period, for a total of 18 collection dates (Extended Data Table 1). At least 1 rRT–PCR-positive sample was identified from 17 out of 18 collection dates, with the majority of positive sample C_t values being below 31 (Fig. 1b). Prevalence estimates varied from 13.5% to 70% across the nine sites (Fig. 1c). The highest prevalence estimates of SARS-CoV-2 were observed at four sites (sites 2, 5, 7 and 9) situated in the northern section of the sampled area, adjacent to urban areas with high human population densities (Fig. 1a, c). Male deer ($\chi^2 = 25.45$, $P < 0.0005$) and heavier deer (Wilcoxon–Mann–Whitney $P = 0.0056$) were more likely to test positive for SARS-CoV-2 (Extended Data Table 2).

Three SARS-CoV-2 lineages identified

We sequenced the complete genome of 14 viruses collected from 6 of the 9 sites, collected at 7 time points spanning from 26 January 2021 to 25 February 2021 (Supplementary Table 1). The deer samples were collected approximately six weeks after the peak of Ohio's 2020–2021 winter epidemic of SARS-CoV-2 in humans, which was dominated by B.1.2 viruses (more than 50% of human viruses) (Fig. 2a, Extended Data Table 3). B.1.2 viruses genetically similar to human viruses were detected in deer at sites 4, 7, 8 and 9 (Fig. 2b). B.1.596, a minor lineage (accounting for around 11% of human viruses), was identified in seven deer samples at site 1, spanning two collection times (2 February and 25 February 2021). A rarer lineage, B.1.582 (present in approximately 1% of human samples), was identified in two deer samples at site 6. No sequences belonging to the Alpha (B.1.1.7) or Delta (B.1.617.2) lineages were identified in the deer samples, as these variants became widespread in the human population only after February 2021.

Six human-to-deer transmission events

Although B.1.2 was identified in deer at 4 sites, our phylogenetic analysis found no evidence of B.1.2 viruses transmitting in deer across sites. Rather, each site experienced a separate human-to-deer transmission

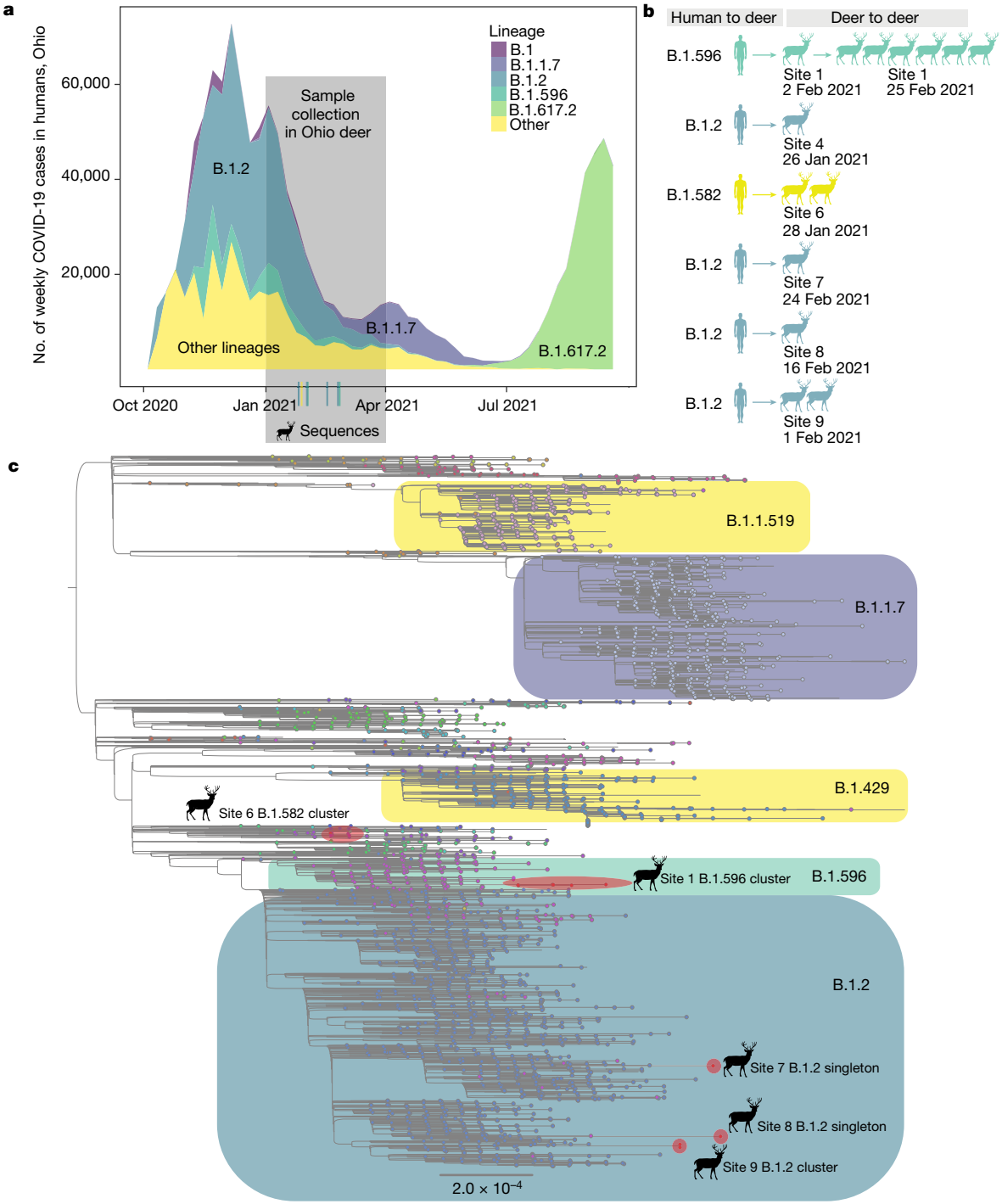

Fig. 2 | Three SARS-CoV-2 lineages identified in white-tailed deer. a, The number of weekly COVID-19 cases in humans from October 2020 to September 2021 in Ohio. Shading indicates the proportion of viruses sequenced each week in Ohio that belong to one of five Pango lineages (or 'other'). **b**, Summary of six human-to-deer transmission events observed in Ohio, with putative deer-to-deer transmission. **c**, Maximum-likelihood tree inferred for SARS-CoV-2 viruses in humans and white-tailed deer in Ohio during January to March 2021. Tips are shaded by Pango lineage and major lineages are boxed, labelled and shaded similar to Fig. 2b. Viruses found in white-tailed deer (clusters or singletons) are shaded red and labelled by location (the B.1.2 virus identified at site 4 is not shown owing to lower sequence coverage). All branch lengths are drawn to scale.

event of a slightly genetically different B.1.2 virus positioned in a different section of the B.1.2 clade on the phylogenetic tree (Fig. 2c). In total, six human-to-deer transmission events were observed: B.1.582 (site 6), B.1.596 (site 1) and B.1.2 (sites 4, 7, 8 and 9). There is a degree of uncertainty about the timing of each viral entry into the deer population, owing to long branch lengths that separate the deer viruses from the ancestral human viruses on the phylogenetic tree. To estimate the timing and location of human-to-deer transmission for the larger cluster of B.1.596 deer viruses, a time-scale Bayesian maximum clade credibility (MCC) tree was inferred using a phylogeographic approach (Fig. 3a). The MCC tree is consistent with human-to-deer transmission occurring geographically in Ohio (posterior probability = 0.98) and temporally during the winter epidemic when viral loads in humans (and the environment) would be peaking. The MCC tree indicates

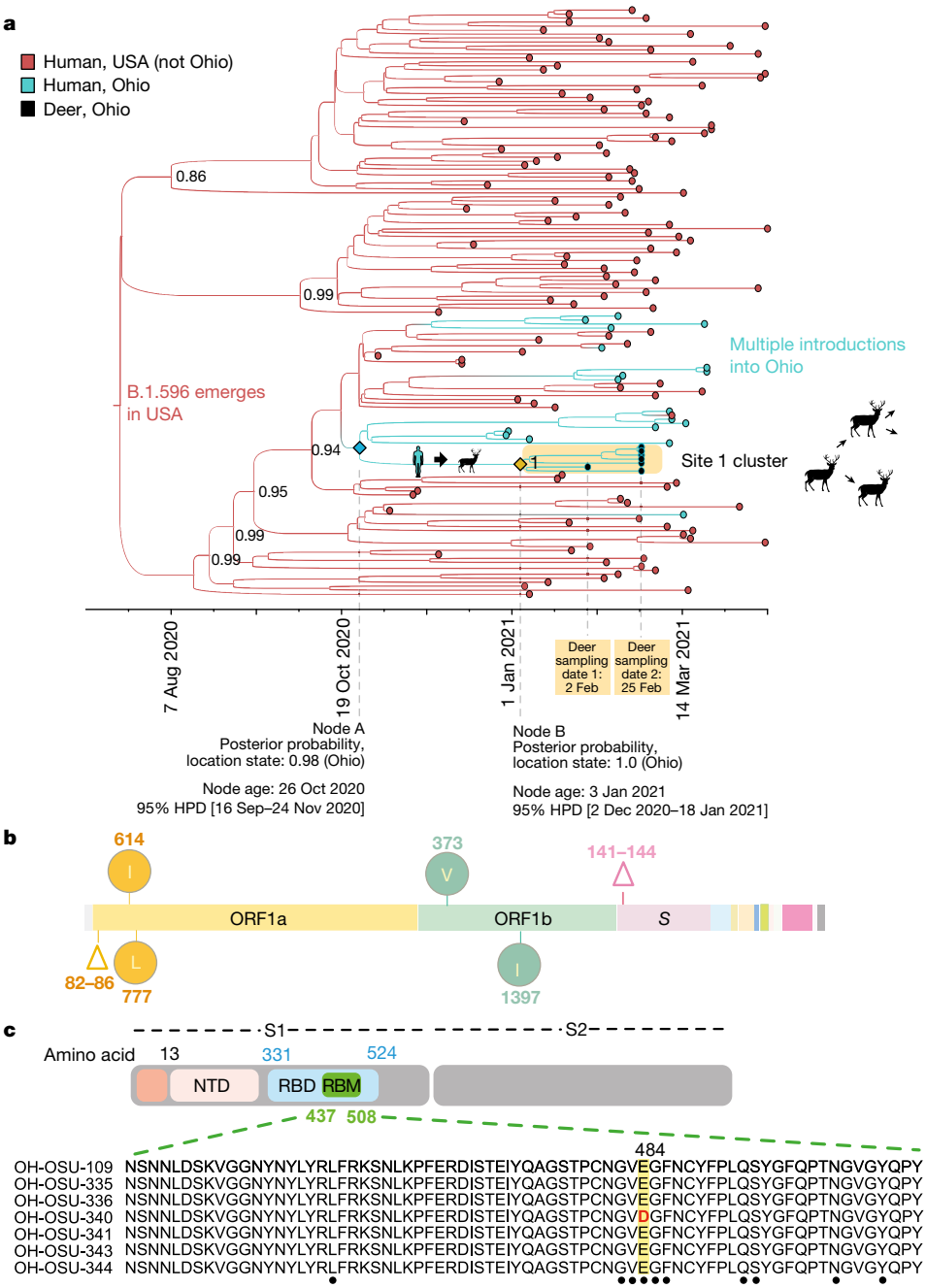

Fig. 3 | Evolution of B.1.596 viruses in white-tailed deer. a, Bayesian time-scale MCC tree inferred for the cluster of 7 B.1.596 viruses identified in white-tailed deer at site 1, the 46 most closely related human B.1.596 viruses, and a random sampling of other B.1.596 viruses observed in the USA during November 2020 to March 2021. Tips are shaded by location state (host species and geography). Branches are shaded by the location state inferred from an ancestral reconstruction. Posterior probabilities are provided for key nodes. Cartoons indicate the host-switch branch where human-to-deer transmission may have occurred, followed by putative deer-to-deer transmission within site 1. The estimated timing and location state probability is provided for key nodes defining the host-switch branch. **b**, Clade-defining amino acid changes observed in all 7 B.1.596 deer viruses are listed. **c**, The E484D substitution in the spike protein's receptor-binding motif (RBM) is shown in one of the B.1.596 deer viruses (OH-OSU-340). NTD, N-terminal domain; RBD, receptor-binding domain.

that B.1.596 viruses were introduced into humans in Ohio multiple times from other US states during the autumn of 2020 and winter of 2020–2021, forming three co-circulating Ohio clades in humans. The largest Ohio clade then seeded the deer outbreak. Deer viruses in this cluster were collected on 2 February and 25 February 2021, and the MCC tree estimates that human-to-deer transmission occurred several weeks, or possibly months, earlier (Fig. 3a). Gaps in sampling in both humans and deer make it difficult to narrow this time estimate further.

Deer-to-deer transmission and evolution

Viable SARS-CoV-2 virus was recovered from two of the deer samples (Extended Data Table 4). Deer-to-deer transmission may have occurred within the three study sites where more than 1 deer sample was sequenced: site 1 (B.1.596), site 6 (B.1.582) and site 9 (B.1.2). Only two viruses were collected from sites 6 and 9, both from the same sampling date (Fig. 2b), limiting what can be inferred about transmission. Instead, our analysis of deer-to-deer transmission and evolution

focused on the larger deer cluster of 7 B.1.596 viruses observed in site 1 that spans two collection dates (Fig. 3a). A number of uncommon amino acid substitutions were observed in all seven deer viruses in this clade (that is, all site 1 sequences) that were not observed in the most closely related human viruses. Five clade-defining mutations were observed in ORF1ab: a five-residue deletion in nsp1 (Δ82–86), nsp2 T434I, nsp2 P597L, nsp12 A382V and nsp13 M474I (numbering in Fig. 3b refers to ORF1a and ORF1b). A clade-defining deletion (Δ141–144) was also observed in the S1 domain of the spike protein in the 7 deer viruses. All 6 clade-defining mutations observed in these deer are uncommon among human viruses (<0.05% frequency globally; https://outbreak.info/).

Uncommon amino acid changes in the spike protein S1 domain also were observed in singleton deer viruses. A B.1.2 virus from site 7 (hCoV-19/deer/USA/OH-OSU-0212/2021) has a substitution in the N-terminal domain of the spike protein (H245Y). A single B.1.596 virus from site 1 (hCoV-19/deer/USA/OH-OSU-0340/2021) has a substitution in the spike protein receptor-binding motif (E484D) (Fig. 3c). Both mutations are relatively rare in humans, being found in less than 0.5% of all SARS-CoV-2 viruses sequenced globally. In experimental studies, viruses with the E484D substitution are less sensitive to neutralization by convalescent sera[26]. The E484D substitution has been detected only in 201 SARS-CoV-2 sequences from humans globally, 71 of which were in the USA, but none of the B.1.596 viruses in humans that were most closely related to the deer virus have this mutation. It is therefore impossible to differentiate whether the E484D mutation arose in an unsampled human virus and was transmitted to deer or arose de novo in deer. Additionally, owing to low availability of sequence data from deer it is not possible to determine whether these spike mutations have been transmitted to other deer.

Discussion

Our finding that white-tailed deer are frequently infected with SARS-CoV-2 viruses raises profound questions about the future trajectory of SARS-CoV-2. The potential establishment of a new reservoir of SARS-CoV-2 viruses in white-tailed deer could open new pathways for evolution, transmission to other wildlife species and potential spillback of novel variants to humans that the human immune system has not previously encountered. SARS-CoV-2 viruses have a high capacity for adaptive evolution when infection rates are high in a community or population. It is therefore concerning that more than one-third of deer in our study tested positive for SARS-CoV-2 by PCR, suggesting an active or recent infection during the major wave the previous winter. A number of mutations were observed in white-tailed deer that occur at very low frequency in humans, including a mutation in the receptor-binding motif. Such mutations could potentially be amplified in a new reservoir host with high infection rates and different constraints on evolution. There is an urgent need to expand monitoring of SARS-CoV-2 viruses in potential wildlife hosts to document the breadth of the problem in white-tailed deer nationally, understand the ecology of transmission and track evolutionary trajectories going forward, including in other potential host species.

The impact of urban sprawl on disease ecology is well documented for Lyme disease and other multihost zoonotic systems that include white-tailed deer, rodents and other species that have become ubiquitous and well adapted in expanding US urban and semi-urban environments, creating opportunities for pathogen exchange. Approximately 30 million free-ranging white-tailed deer are distributed broadly across urban, suburban, and rural environments in the USA, and can live at densities of greater than 45 deer per square mile in some areas[27]. Ohio is home to more than 700,000 free-ranging white-tailed deer[28] and another 440 commercial deer farms[29]. Estimates of deer density in and around our sites range from approximately 8 km^{-2} to upwards of 30 km^{-2}. There are no deer farms in the study area and public feeding of deer is prohibited. There is ample forage available around urban and suburban residences in gardens and plantings, drawing deer into close proximity with humans and their companion animals. Therefore, is unsurprising that deer in urban sites were at higher risk for infection in our study. Urban settings provide ample opportunities for deer to have direct and indirect contact with human-contaminated sources (for example, trash, backyard feeders, bait stations and wildlife hospitals) that could serve as a pathway for viral spillover into wildlife. Additionally, urban and suburban environments include waterways that could be contaminated by multiple sources[18,30]. Viable SARS-CoV-2 is shed in human stool. SARS-CoV-2 RNA and has also been detected in wastewater[31,32] and urban runoff[33], although the infectivity of SARS-CoV-2 from these sources is undetermined. The recent detection of genetically distinct SARS-CoV-2 virus fragments in New York City wastewater introduces an intriguing hypothesis that SARS-CoV-2 could be transmitting cryptically in rodents[34]. However, although sensitive techniques for detecting viral RNA in wastewater have vastly improved, providing a potentially useful tool for early detection of outbreaks, isolating or whole-genome sequencing viruses to characterize their genetic diversity remains challenging.

A major outstanding question is how the virus is transmitted between deer. Deer are social animals that live in small herds and frequently touch noses. It is unclear whether baiting the deer before collection contributed the increased frequency of SARS-CoV-2 in this study, but concentrating deer with bait could potentially have facilitated pathogen transmission through a population. However, baiting is regularly used in deer population-management programmes and the practice is commonly employed by deer hunters, which makes understanding the effect of baiting on SARS-CoV-2 transmission in free-ranging deer paramount for future studies. The increased rate of infection in males in this study could reflect sex-linked differences in behaviour that increase disease transmission. The higher prevalence of chronic wasting disease and tuberculosis in male white-tailed deer is attributed to larger male home ranges, increased movement and contact with other deer during breeding season (autumn–winter), and dynamic male social group composition and size[35]. Deer may experience high levels of viraemia and shedding that may be conducive to environmental or aerosol transmission. Another question is whether deer experience clinical disease and whether clinical signs such as sneezing or nasal discharge increase the risk of transmission. Two previous experimental studies reported only subclinical infections in white-tailed deer challenged with SARS-CoV-2, but these studies had very small sample sizes[14,23].

Although extensive measures were taken to prevent cross-contamination during sample collection and testing, the nature of field work makes it impossible to completely exclude the possibility. Cross-contamination during sample collection would not invalidate the detection of SARS-CoV-2 in white-tailed deer, but would artificially inflate prevalence estimates. However, the extent of genomic diversity among the sequences recovered on a single sampling day (for example, site 1, sampling 2) indicates cross-contamination during sample collection was probably minimal.

Although our study was limited to northeast Ohio, these findings have implications for other US states, including Michigan, Pennsylvania, New York and Illinois, where high rates of exposure to SARS-CoV-2 in white-tailed deer have been reported on the basis of serology[24]. Serological assays are notoriously difficult to interpret and many animal health experts had hoped that those results were an artefact. Moreover, the detection of antibodies does not prove active infection. However, the present study suggests that the antibodies observed in deer in other US states may have arisen from active infection, and that the true extent of infections may remain underestimated.

Moreover, it is worth noting that white-tailed deer are a relatively convenient surveillance target because of their abundance and accessibility. The detection of SARS-CoV-2 in free-ranging white-tailed deer naturally raises the question of whether less accessible species are also being infected through viral spillover from humans, which calls for broader surveillance efforts.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-021-04353-x.

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Methods

Sample collection

Between January and March 2021, 360 free-ranging white-tailed deer originating from 9 study sites in northeast Ohio (USA) were euthanized as part of a deer population-management programme. Collection occurred at locations that were baited with whole kernel corn for up to two weeks prior to each culling session, and additional deer were collected opportunistically when they were observed away from the bait on a culling session day. In the field, once a deer was collected, the head was wrapped in a plastic bag and an identification tag was attached to a leg. Each day of the programme, collected deer carcasses were transported to a central processing point where samples were collected. All samples were collected by one experienced veterinarian who wore a facemask and gloves that were changed or washed between each sample. 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. Samples were collected post mortem, which was exempt from oversight by The Ohio State University Institutional Animal Care and Use Committee.

Diagnostic testing

Samples were initially tested using the Charité/Berlin (WHO) assay[36]. Viral RNA was extracted from 200 µl of BHIB using Omega Bio-tek Mag-Bind Viral DNA/RNA kit (catalogue (cat.) no. M6246-03). Xeno Internal Control (Life Technologies cat. no. A29763) was included in the extraction to ensure the accuracy of negative results. Five microlitres of extracted RNA was added to Path-ID MPX One-Step Kit master mix (Life Technologies cat. no. 4442135) containing 12.5 µl 2× Multiplex RT-PCR buffer, 2.5 µl enzyme mix, 1.5 µl nuclease free water, 4.5 µl E assay primer/probe panel (Integrated DNA Technologies cat. no. 1006804), and 1 µl XENO VIC Internal Control Assay (Life Technologies cat. no. A29765) for each sample. The cycling parameters for the rRT–PCR were 48 °C for 10 min, 95 °C for 10 min, 45 cycles of 95 °C 15 s and 58 °C 45 s. Samples with a cycle threshold (C_t) of ≤40 were considered positive. If the E assay was positive, the RdRp confirmatory and discriminatory assays were completed using the above master mix formulation and thermocycler parameters, replacing the E assay primer–probe panel with the confirmatory and discriminatory primer–probe panel (Integrated DNA Technologies cat. no. 10006805 and 10006806). The RNA from all samples that tested positive with the E assay, was retested with the CDC rRT–PCR protocol[37]. Samples that were 2019-nCoV N1 and N2 positive were classified as presumptive positive. A subset of presumptive positive samples was selected for retesting, in which RNA was re-extracted from original samples to verify the rRT–PCR result.

Genomic sequencing

Original sample material for 76 representative presumptive positive samples were sent to the National Veterinary Services Laboratories (NVSL) for confirmatory rRT–PCR testing using the CDC protocol and whole-genome sequencing. Viral RNA was amplified by PCR[38] and cDNA libraries were prepared using the Nextera XT DNA Sample Preparation Kit according to manufacturer instructions. Sequencing was performed using the 500-cycle MiSeq Reagent Kit v2. Sequences were assembled using IRMA v.0.6.7 and DNAStar SeqMan NGen v.14.0.1. Additional sequencing was attempted at Ohio State's Applied Microbiology Services Laboratory using a modified ARTIC V3 method (https://www.protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye). Extracted RNA was reverse transcribed and amplified by PCR with the ARTIC SARS-CoV-2 FS Library Prep Kit (New England Biolabs) according to the manufacturer's recommended protocol. Amplified products were converted to Illumina sequencing libraries using the RNA Prep with Enrichment (L) Tagmentation Kit protocol (Illumina) with unique dual indexes and 10 cycles of TagPCR. Sequencing libraries were pooled and quantified using ProNex NGS Library Quant Kit (NG1201, Promega). The 650 pM libraries were loaded on P2 sequencing cartridges and analysed with the NextSeq2000 (Illumina) with 2 × 101 bp cycles. Data were transmitted to the BaseSpace Cloud platform (Illumina) and converted to FASTQ file format using DRAGEN FASTQ Generation v3.8.4 (Illumina). DRAGEN COVID Lineage app v.3.5.3 (Illumina) was used to align sequence data and produce quality metrics and consensus genome sequences.

Data analysis

Pangolin v.3.1.11, 2021-09-17 was used to assign lineage[39,40]. Prevalence was estimated using the number of presumptive positive nasal swabs based upon the final CDC rRT–PCR results. Prevalence estimates, confidence intervals, and other descriptive statistics were calculated using STATA 14.2 (StataCorp).

Virus isolation

In brief, at the NVSL, the samples were diluted between 1:2 and 1:3 in minimum essential medium with Earle's balanced salt solution (MEM-E). Vero 76 cells that were mycoplasma-free were inoculated with 1.5 ml diluted sample material and adsorbed for 1 h at 37 °C. After adsorption, a replacement medium containing 2 µg ml^{-1} *N*-*p*-tosyl-L-phenylalanine chloromethyl ketone-treated trypsin was added, and cells were incubated at 37 °C for up to 7 days. Cell cultures with exhibiting no cytopathic effects (CPE) were frozen, thawed, and subjected to two blind passages, inoculating the fresh cultures with those lysates as described above. At the end of two blind passages or upon observation of CPE, cell culture material was tested by rRT–PCR for SARS-CoV-2 using the CDC N1 and N2 primer and probe sets.

Phylogenetic analysis

First, a background dataset was compiled from GISAID that included all SARS-CoV-2 sequences available from humans in Ohio, USA during the study period (1 January to 31 March 2021), downloaded on 27 September 2021 (n = 4,801 sequences). To our knowledge, these are the first SARS-CoV-2 viruses sequenced from white-tailed deer globally and no additional sequences from white-tailed deer were available in any public repository for comparison. Pangolin was used to assign a lineage to each human virus. In total, 102 lineages were identified in this dataset, with the most common being B.1.2 (n = 1,766), B.1.1.7 (n = 833), B.1.1.519 (n = 411), B.1.429 (n = 307) and B.1.596 (n = 274). The dataset was aligned using NextClade with Wuhan-Hu-1 as a reference. The alignment was manually trimmed at the 5′ and 3′ ends. The final alignment included only coding regions and was manually edited to be in frame, with stop codons present only at the terminus of genes. A phylogenetic tree was inferred from this dataset using maximum-likelihood methods available in IQ-TREE version 1.6.12 with a GTR + G model of nucleotide substitution and 1,000 bootstrap replicates, using the high-performance computational capabilities of the Biowulf Linux cluster at the National Institutes of Health (http://biowulf.nih.gov). The inferred tree was visualized in FigTree v.1.4.4. Outlier sequences were removed with long branch lengths and incongruence between genetic divergence and sampling date, as assessed using TempEst v.1.5.3, typically arising from 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. To examine the evolutionary origins of the cluster of 7 B.1.596 viruses obtained from deer at site 1 in more granular detail, a second phylogenetic tree was inferred that included all B.1.596 sequences available globally from NCBI's GenBank (n = 5,586), nearly all (99.8%) from the USA, using similar methods as above. For purposes of visualization a separate phylogenetic tree was inferred that was limited to the sub-clade of B.1.596 viruses (n = 46) most closely related to the 7 deer viruses. This clade, plus 100 viruses randomly sampled from other sections of the tree as background, was used in a subsequent

Bayesian phylogeographic analysis. A time-scaled Bayesian analysis using the Markov chain Monte Carlo (MCMC) method was performed the BEAST v.1.10.4 package[41], again using the Biowulf Linux cluster. A relaxed uncorrelated lognormal (UCLN) molecular clock was used with a flexible Bayesian skyline population, and a general-time reversible (GTR) model of nucleotide substitution with gamma-distributed rate variation among sites. Each sample was assigned to one of three categories based on host and geography: (1) viruses collected in humans in all US states except Ohio, (2) viruses collected in humans in Ohio, and (3) viruses collected in deer in Ohio. The MCMC chain was run separately three times for each of the datasets for at least 100 million iterations with subsampling every 10,000 iterations, using the BEAGLE 3 library to improve computational performance[42]. All parameters reached convergence, as assessed visually using Tracer v.1.7.1, with statistical uncertainty reflected in values of the 95% highest posterior density (HPD). At least 10% of the chain was removed as burn-in and runs were combined using LogCombiner v1.10.4 and a MCC tree was summarized using TreeAnnotator v.1.10.4 and visualized in FigTree v.1.4.4. The NVSL vSNP pipeline ((https://github.com/USDA-VS/vSNP) was applied for SNP based phylogenetic analysis using Wuhan-Hu-1 (NC_045512) as a reference.

Epidemiological data

The epidemiological curve of SARS-CoV-2 cases in Ohio from April 2020 to September 2020 was generated using the number of daily reported COVID-19 cases in the state of Ohio (all age groups), available from the US Centers for Disease Control and Prevention (https://data.cdc.gov/Case-Surveillance/COVID-19-Case-Surveillance-Public-Use-Data-with-Ge/n8mc-b4w4). All SARS-CoV-2 genetic sequences from Ohio were downloaded from GISAID on 8 October 2021 (n = 18,052) to estimate the proportion of viruses belonging to different Pango lineages during each week of the epidemic. To account for the intensity of surveillance not being even over time the number of viruses per lineage per week was normalized against the epidemiological curve derived from COVID-19 case counts and visualized using R. To further minimize biases only sequences categorized in the GISAID submission as obtained using a 'baseline surveillance' sampling strategy were included in the analysis. The dataset was further trimmed to include only submissions with complete collection dates and sufficient coverage to assign a Pango lineage, resulting in a final dataset of 9,947 sequences from Ohio. For simplicity sub-lineages of B.1.617.2 (for example, AY.3) were consolidated into the Delta category and sub-lineages of B.1.1.7 (for example, Q.3) were consolidated into the Alpha category. Baseline surveillance data before 20 December 2020 was too thinly sampled to reliably estimate the proportion of viruses from different lineages from this time period, so a second figure was generated using all available sequence data. As the proportions of Pango lineages over time proved to be very similar in the baseline data and the complete dataset, the larger dataset that dated back to October 2020 was used in the final figure.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Sequence data from the 14 SARS-CoV-2 viruses from white-tailed deer sequenced in this study are available at NCBI Sequence Read Archive (SRA) under accessions SRR17187542, SRR17187543, SRR17187544, SRR17187545, SRR17187546, SRR17187547, SRR17187548, SRR17187549, SRR17187550, SRR17187551, SRR17187552, SRR17187553, SRR17187554 and SRR17187555 and assembled genome sequences are on GISAID (Extended Data Table 4).

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Author contributions Conceptualization: V.L.H., P.M.D., J.M.N., D.L., S.G., L.S., S.A.F. and A.S.B. Methodology: V.L.H., P.M.D., J.M.N., S.A.F., M.I.N. and A.S.B. Investigation: P.M.D., J.G., C.M., D.H., M.E., R.M.T., M.L.K., K.L., S.R.-A., M.T. and A.S.B. Visualization: M.I.N., D.S.M. and A.S.B. Funding acquisition: V.L.H., J.M.N., J.W., S.A.F. and A.S.B. Project administration: V.L.H., P.M.D., J.M.N. and A.S.B. Supervision: V.L.H., P.M.D., J.G., M.T. and A.S.B. Writing, original draft: V.L.H., M.I.N., D.S.M., M.L.K., S.A.F. and A.S.B. Writing, review and editing: P.M.D., C.M., D.H., J.G., J.M.N., D.L., J.W., S.G., L.S., M.E., R.T., K.L., S.R.-A. and M.T.

Competing interests The authors declare no competing interests.

Additional information

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Extended Data Table 1 | SARS-CoV-2 prevalence stratified by site and sampling date.

	Date of collection	Samples	Positive	Estimated Prevalence	Lower 95% CI	Upper 95% CI
Site 1	**2021-02-02**	41	2	5%	1%	17%
	2021-02-25	33	8	24%	11%	42%
Site 2	2021-1-27	4	3	75%	19%	99%
	2021-03-03	16	11	69%	41%	89%
Site 3	2021-03-02	24	8	33%	16%	55%
Site 4	**2021-01-26**	14	2	14%	2%	43%
	2021-02-17	16	4	25%	7%	52%
Site 5	2021-01-25	16	8	50%	25%	75%
	2021-02-09	8	5	63%	24%	91%
	2021-03-08	6	4	67%	22%	96%
Site 6	**2021-01-28**	33	3	9%	2%	24%
	2021-03-04	15	7	47%	21%	73%
Site 7	2021-02-22	20	5	25%	9%	49%
	2021-02-23	19	8	42%	20%	67%
	2021-02-24	50	36	72%	58%	84%
Site 8	**2021-02-16**	13	3	23%	5%	54%
Site 9	**2021-02-01**	22	12	55%	32%	76%
	2021-03-01	10	0	0%	0%	31%*

Sample collection dates for all nine sites are shown indicating the number of nasal swabs collected from white-tailed deer on each date and the number of those swabs that were screened positive for SARS-CoV-2 using the WHO and Centers for Disease Control and Prevention rRT–PCR protocols in series. Overall estimated prevalence is shown with 95% confidence interval estimates (Clopper-Pearson exact). The 7 collection dates from which genetic sequences were obtained are in bold text.

Extended Data Table 2 | Covariate data recorded for each deer sample

Categorical

Covariate	Category	Samples	Positive (%)	Odds Ratio	Chi2	P-Value
Sex				3.10	25.45	<0.0005
	Male	149	76 (51)			
	Female	211	53 (25)			
Cull method				0.82	0.72	0.395
	Treestand	119	39 (33)			
	Opportunistic	241	90 (37)			

Continuous

Covariate	Samples	Negative mean (std)	Positive mean (std)	Difference	z (Mann-Whitney)	P-Value
Mass (Kg)	360	52.76 (13.48)	56.73 (13.93)	3.972	2.77	0.0056
Age (years)	357	2.11	2.05	-0.053	-0.035	0.9717

rRT–PCR positive samples are shown by group for categorical variables, p-values shown from Pearson's Chi2. The mean of each continuous variable is show for rRT–PCR positive and negative deer respectively, p-values shows from Wilcoxon-Mann-Whitney rank-sum test (two-sided). Age was not recorded for 3 deer which tested negative for SARS-CoV-2.

Extended Data Table 3 | Proportion of SARS-CoV-2 viruses identified in Ohio in humans during January 1 – February 28, 2021

Rank	Lineage	Percentage	Number
1	B.1.2	56.3%	462
2	B.1.596	8.3%	68
3	B.1.1.519	7.1%	58
4	B.1.429	3.4%	28
5	B.1.1.7	3.0%	25
6	B.1.234	2.7%	22
7	P.2	2.4%	20
8	B.1.311	1.6%	13
9	B.1.427	1.5%	12
10	B.1.400	1.2%	10
11	B.1.582	1.2%	10
12	B.1.1	1.1%	9
13	B.1.243	1.1%	9
14	B.1	0.7%	6
15	B.1.568	0.7%	6
16	B.1.595	0.6%	5
17	B.1.1.337	0.5%	4
18	B.1.240	0.5%	4
19	B.1.448	0.5%	4
20	B.1.1.207	0.4%	3
21	B.1.1.434	0.4%	3
22	B.1.265	0.4%	3
23	B.1.361	0.4%	3
24	B.1.438.4	0.4%	3
25	B.1.577	0.4%	3
26	B.1.588	0.4%	3
27	B.1.1.265	0.2%	2
28	B.1.298	0.2%	2
29	B.1.517	0.2%	2
30	B.1.565	0.2%	2
31	Q.8	0.2%	2
32	B.1.1.291	0.1%	1
33	B.1.1.316	0.1%	1
34	B.1.1.348	0.1%	1
35	B.1.110.3	0.1%	1
36	B.1.139	0.1%	1
37	B.1.324	0.1%	1
38	B.1.346	0.1%	1
39	B.1.404	0.1%	1
40	B.1.409	0.1%	1
41	B.1.493	0.1%	1
42	B.1.503	0.1%	1
43	B.1.564	0.1%	1
44	C.23	0.1%	1
45	C.31	0.1%	1

Only viruses categorized as collected for baseline surveillance are included.

Extended Data Table 4 | Whole genome sequence data

Virus name	Site	Collection date	GenBank SRA Accession	GISAID Accession	Submitting laboratory
hCoV-19/deer/USA/OH-OSU-0025/2021	4	2021-01-26	SRR17187543	EPI_ISL_4878314	Ohio State
hCoV-19/deer/USA/OH-OSU-0057/2021	6	2021-01-28	SRR17187550	EPI_ISL_4847029	NVSL
hCoV-19/deer/USA/OH-OSU-0058/2021	6	2021-01-28	SRR17187551	EPI_ISL_4847030	NVSL
hCoV-19/deer/USA/OH-OSU-0078/2021	9	2021-02-01	SRR17187544	EPI_ISL_4878315	Ohio State
hCoV-19/deer/USA/OH-OSU-0079/2021	9	2021-02-01	SRR17187552	EPI_ISL_4847031	Ohio State
hCoV-19/deer/USA/OH-OSU-0109/2021	1	2021-02-02	SRR17187545	EPI_ISL_4878316	NVSL
hCoV-19/deer/USA/OH-OSU-0141/2021	8	2021-02-16	SRR17187553	EPI_ISL_4847032	NVSL
hCoV-19/deer/USA/OH-OSU-0212/2021	7	2021-02-24	SRR17187542	EPI_ISL_4847033	NVSL
hCoV-19/deer/USA/OH-OSU-0335/2021	1	2021-02-25	SRR17187546	EPI_ISL_4878317	NVSL
hCoV-19/deer/USA/OH-OSU-0336/2021	1	2021-02-25	SRR17187547	EPI_ISL_4878318	NVSL
hCoV-19/deer/USA/OH-OSU-0340/2021	1	2021-02-25	SRR17187548	EPI_ISL_4878319	NVSL
hCoV-19/deer/USA/OH-OSU-0341/2021	1	2021-02-25	SRR17187549	EPI_ISL_4878320	NVSL
hCoV-19/deer/USA/OH-OSU-0343/2021	1	2021-02-25	SRR17187554	EPI_ISL_4878321	NVSL
hCoV-19/deer/USA/OH-OSU-0344/2021	1	2021-02-25	SRR17187555	EPI_ISL_4878322	Ohio State

Sequences generated from original sample nasal swabs collected from white-tailed deer as a part of this study are shown with GISAID and NCBI Sequence Read Archive (SRA) accession numbers, collection date, site of collection, and the submitting laboratory indicating if the sequencing was completed at The Ohio State University Applied Microbiology Services Laboratory (Ohio State) or USDA National Veterinary Services Laboratories (NVSL). The two samples from which viable SARS-CoV-2 was isolated are denoted with bold text.

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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 v1.10.4package (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.

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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, 20201-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-CoV2-2 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

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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 pend and paper, and they collected a nasal swab into BHIB. Samples were then shipped to Andrew Bowman where Jacqueline Nolting, Chrisopher 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? ☒ Yes ☐ 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	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ 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 (Odocoileus virginianus) 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.