



## Communication

# Serological Evidence That SARS-CoV-2 Has Not Emerged in Deer in Germany or Austria during the COVID-19 Pandemic

Andres Moreira-Soto <sup>1</sup>, Christian Walzer <sup>2,3</sup>, Gábor Á. Cziráj <sup>4</sup>, Martin H. Richter <sup>5</sup>, Stephen F. Marino <sup>5</sup>, Annika Posautz <sup>3</sup>, Pau De Yebra Rodo <sup>4</sup>, Gayle K. McEwen <sup>4</sup>, Jan Felix Drexler <sup>1,\*,†</sup> and Alex D. Greenwood <sup>4,6,\*,†</sup>

<sup>1</sup> Institute of Virology, Charité-Universitätsmedizin Berlin, Humboldt-Universität zu Berlin, Berlin Institute of Health, 10117 Berlin, Germany; andres.moreira-soto@charite.de

<sup>2</sup> Wildlife Conservation Society, Bronx, NY 10460, USA; cwalzer@wcs.org

<sup>3</sup> Research Institute of Wildlife Ecology (FIWI), A-1160 Vienna, Austria; annika.posautz@vetmeduni.ac.at

<sup>4</sup> Department of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research, 10315 Berlin, Germany; czirjak@izw-berlin.de (G.Á.C.); rodo@izw-berlin.de (P.D.Y.R.); mcewen@izw-berlin.de (G.K.M.)

<sup>5</sup> Bundesinstitut für Risikobewertung (BfR), 10589 Berlin, Germany; martin.richter@bfr.bund.de (M.H.R.); stephen-francis.marino@bfr.bund.de (S.F.M.)

<sup>6</sup> Department of Veterinary Medicine, Freie Universität Berlin, 14163 Berlin, Germany

\* Correspondence: felix.drexler@charite.de (J.F.D.); greenwood@izw-berlin.de (A.D.G.); Tel.: +49-030-450-525-092 (J.F.D.); +49-030-5168-255 (A.D.G.)

† These authors contributed equally to this work.



**Citation:** Moreira-Soto, A.; Walzer, C.; Cziráj, G.Á.; Richter, M.H.; Marino, S.F.; Posautz, A.; De Yebra Rodo, P.; McEwen, G.K.; Drexler, J.F.; Greenwood, A.D. Serological Evidence That SARS-CoV-2 Has Not Emerged in Deer in Germany or Austria during the COVID-19 Pandemic. *Microorganisms* **2022**, *10*, 748. <https://doi.org/10.3390/microorganisms10040748>

Academic Editor:  
Charalambos Billinis

Received: 11 March 2022

Accepted: 29 March 2022

Published: 30 March 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Abstract:** Spillover of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) to North American white-tailed deer (*Odocoileus virginianus*) has been documented. However, it is unclear if this is a phenomenon specific to North American deer or is a broader problem. We evaluated pre and pandemic exposure of German and Austrian deer species using a SARS-CoV-2 pseudoneutralization assay. In stark contrast to North American white-tailed deer, we found no evidence of SARS-CoV-2 exposure.

**Keywords:** COVID-19; SARS-CoV-2; roe deer; red deer; fallow deer; hunting

## 1. Introduction

The COVID-19 pandemic caused by the SARS-CoV-2 virus has spread from humans to domestic animals, captive animals, and free ranging wildlife [1–6]. In some cases, the result was the spillback of a novel SARS-CoV-2 variant to humans, making new wildlife reservoirs a clear threat to human health. Spillover is of particular concern in North America where there is strong evidence that white-tailed deer (*Odocoileus virginianus*) have been infected in the U.S. and Canada with the prevalence reaching 82.5% [3]. White-tailed deer may now represent a reservoir in which SARS-CoV-2 may evolve and potentially spill back to humans with unpredictable health consequences. White-tailed deer are social animals that often form large herds, are important game species, and often live in peri-urban and urban environments, making them unsurprising candidates as a viral reservoir species for pathogens with zoonotic potential. However, it is unclear how generalized spillback is in deer. In Europe, multiple species of deer are heavily managed and hunted but hunting legislation and management practices differ substantially between the U.S. and Europe, within Europe, and even within individual countries and regions. Different deer species may also vary in the sequence of their angiotensin converting enzyme-2 (ACE2) receptor (the receptor with which the SARS-CoV-2 virus spike protein interacts), which could hinder or promote infection [7].

To assess whether SARS-CoV-2 spillover has occurred in Europe, we applied a SARS-CoV-2 pseudoneutralization assay to pandemic-collected sera from the three main species of European cervids, namely red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and

fallow deer (*Dama dama*). Samples were collected from Germany and Austria, two countries with long deer management traditions. We also compared the ACE2 receptor sequences for all deer and related bovids in the databases including roe and red deer to determine if they have amino acid changes that would be consistent with resistance to infection with SARS-CoV-2.

## 2. Materials and Methods

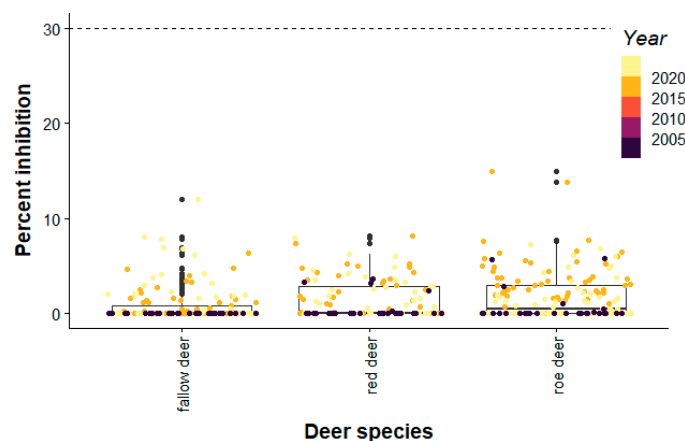
Sera were collected from red deer (*Cervus elaphus*) (n = 67), roe deer (*Capreolus capreolus*) (n = 97), and fallow deer (*Dama dama*) (n = 68) between January 2020 and December 2021 (Tables 1 and S1).

**Table 1.** Number and origin of samples from each deer species analyzed for anti-SARS-CoV-2 antibodies.

	Germany		Austria
	Pre-Pandemic	Pandemic	Pandemic
Roe deer	71	97	
Red deer	45	16	51
Fallow deer	85	68	
Total	201	181	51

A SARS-CoV-2 surrogate virus neutralization test (sVNT; GenScript, Piscataway, NJ, USA), which measures the antibody-mediated inhibition of SARS-CoV-2 receptor-binding domain (RBD)-ACE2 interaction, was used to test the samples [7]. Briefly, equal volume of deer sera was pre-incubated with HRP-conjugated RBD for 30 min at 37 °C, followed by adding it to an ACE2-coated ELISA plate for 15 min at room temperature. A colorimetric signal was developed using TMB substrate and stopped with 1 M HCl. Absorbance values at 450 nm were acquired using a microplate reader. This test achieves 99.93% specificity and 95–100% sensitivity and the species-independent performance has been validated in rabbit, ferret, mouse, cat, dog, hamster, and alpaca serums [8,9]. The same assay was employed in North American white-tailed deer and by the U.S. in Brazilian cats with high seroprevalence [2,10], corroborating the robustness of this testing.

Sample size calculations required a minimum of 81–138 animals, assuming seroprevalences ranged from 30% to 90%, there was a large population size, and 95% confidence intervals (calculations were performed using the package epiDisplay version 3.5.0.1 in R version R-4.1.3 (.r-project.org/)). To assess assay specificity, we further tested pre-pandemic sera from Germany (n = 201) (Table 1). The assay cutoff for the cPass SARS-CoV-2 Neutralization Antibody Detection Kit is 30% inhibition (Figure 1).



**Figure 1.** Results of the pseudoneutralization point assay are shown by year and deer species. Horizontal dashed line indicates the assay cutoff of the cPass SARS-CoV-2 Neutralization Antibody Detection Kit as 30% inhibition.

A full multiple-sequence alignment of the ACE2 protein for the species is characterized in [7], and additional deer species are included from GenBank (GCA\_000751575.1, XP\_037678579.1, XP\_012949915.3, QEQ50331.1, KAB0345583.1, XP\_043752042.1, and XP\_036696353.1). Alignments were performed using ClustalW Multiple Alignment in BioEdit version 7.2.5 (Figures 2 and S1).

	9	22	31	80	91	101	102	107	109	115
<i>Odocoileus virginianus</i>	L	E	K	A	L	Q	Q	V	S	R
<i>Capreolus capreolus</i>	.	.	.	.	.	.	.	.	X	X
<i>Cervus elaphus</i>	.	.	N	.	.	.	.	.	.	.
<i>Muntiacus muntjak</i>	.	.	.	.	F	.	.	.	.	.
<i>Oryx dammah</i>	.	G	.	R	.	H	.	.	T	.
<i>Nanger dama</i>	-	-	-	-	-	-	-	-	-	-
<i>Bubalus bubalis</i>	.	.	.	.	.	.	H	A	.	Q
<i>Bos taurus</i>	S	.	.	.	.	.	H	A	.	.
	121	122	159	195	209	211	228	250	251	270
<i>Odocoileus virginianus</i>	N	T	N	N	T	E	N	D	T	D
<i>Capreolus capreolus</i>	.	.	S	X	.	.	H	.	.	X
<i>Cervus elaphus</i>	.	.	.	.	.	.	H	.	.	.
<i>Muntiacus muntjak</i>	.	.	.	.	.	.	H	.	A	.
<i>Oryx dammah</i>	.	.	.	.	G	G	R	.	.	.
<i>Nanger dama</i>	-	-	-	-	-	-	-	-	-	-
<i>Bubalus bubalis</i>	.	K	.	.	.	G	H	H	.	.
<i>Bos taurus</i>	.	K	.	.	.	G	R	H	.	.
	287	294	297	299	310	320	323	330	392	426
<i>Odocoileus virginianus</i>	F	V	K	K	K	S	S	D	D	E
<i>Capreolus capreolus</i>	.	.	E	E	.	.	.	.	N	.
<i>Cervus elaphus</i>	.	.	.	E	.	G	.	.	N	.
<i>Muntiacus muntjak</i>	.	I	E	E	R	.	.	.	N	.
<i>Oryx dammah</i>	E	.	.	.	.	.	Y	.	N	A
<i>Nanger dama</i>	-	-	-	-	.	.	Y	E	N	A
<i>Bubalus bubalis</i>	E	.	E	E	.	.	Y	.	N	A
<i>Bos taurus</i>	E	.	E	E	.	.	Y	.	N	A
	430	471	476	535	560	573	601			
<i>Odocoileus virginianus</i>	Y	E	E	N	S	S	R			
<i>Capreolus capreolus</i>	.	.	.	.	.	.	.			
<i>Cervus elaphus</i>	.	.	Q	K	.	.	K			
<i>Muntiacus muntjak</i>	.	.	Q	.	.	.	.			
<i>Oryx dammah</i>	.	Q	.	K	R	.	.			
<i>Nanger dama</i>	.	Q	.	K	R	N	.			
<i>Bubalus bubalis</i>	H	Q	.	K	R	N	.			
<i>Bos taurus</i>	H	Q	.	K	R	N	.			

**Figure 2.** Multiple sequences the ACE2 protein were performed with the species characterized in [7], and additional deer species included from GenBank (GCA\_000751575.1, XP\_037678579.1, XP\_012949915.3, QEQ50331.1, KAB0345583.1, XP\_043752042.1, XP\_036696353.1). The full alignment is in Supplementary Figure S1. The figure only shows deer and related bovids and only positions where there are amino acid changes relative to white-tailed deer (*Odocoileus virginianus*). Alignments were performed using ClustalW Multiple Alignment in BioEdit version 7.2.5. The names of species highlighted in bold correspond to species known to be susceptible to SARS-CoV-2 and the amino acid position highlighted in orange is a hot spot for SARS-CoV-2 S-binding [7]. Ambiguities in database sequences are designated as X. Dots represent identity to the reference and dashes represent missing sequence information.

### 3. Results

All positive and negative controls were in the 450 nm range stated in the manufacturer's instructions. In stark contrast to North American white-tailed deer, no pre- or pandemic deer sera collected tested positive for SARS-CoV-2 antibodies (Figure 1, Tables 1 and S1). None of the samples approached the assay cutoff.

Alignment of the ACE2 receptor among deer and related bovids showed that apart from K-N substitution in the red deer relative to white-tailed deer, none of the sequenced deer species had any substitutions that distinguished them from SARS-CoV-2-susceptible cervid species or other susceptible mammals (Figures 2 and S1).

#### 4. Discussion

The contrast between our results and the multiple reports of high prevalence infection in North American white-tailed deer may have several explanations. First, a single relevant amino acid change was observed in red deer at position 31 (K-N) in the ACE2 receptor compared to other deer and bovids. While this could, in principle, explain infection resistance in red deer, roe deer were identical at all sites of import to white-tailed deer, yet were not infected in our study. It is not clear if this difference would make red deer resistant as other susceptible species vary at this position [7] (Figure 2). For sample size calculations, we assumed equal seroprevalences in all deer species. Since there is no preliminary data on susceptibility to SARS-CoV-2 in European deer species, alignment of ACE2 receptor residues amongst cervids suggest similar susceptibility as white-tailed deer, and all European deer share comparable habitats leading to probable similar contact with SARS-CoV-2. In addition, approximately 40% of tested white-tailed deer in the U.S. were positive using the same test [2]. Thus, even if infection occurs at a very low prevalence, the differences between North America and central Europe remain striking.

In Europe, the historic feudal systems, land use, and land allocation practices shaped hunting traditions. Across Germany, Austria and a large part of Switzerland, hunting is implemented using a district-based hunting system (*Revier*) and wildlife belongs to no one (*res nullius*) [11]. In contrast, in North America, wildlife is considered a public resource managed by the government regardless of the land or water where wildlife lives and is tightly linked to wildlife conservation (*res communis*). This distinction is relevant to the extent to which government bodies can control and influence the management of wildlife and hunting practices. Therefore, in Europe, deer management is more local than in North America [12].

Across the U.S. and Canada, the deer hunting season is generally short, lasting three to four months, while in Europe it varies widely, from less than four weeks in some areas of Switzerland to 213–300 or more days in Austria and Germany [13]. Hunting practices vary across the two regions; in North America, deer hunting over bait and attractants is allowed in over 20 states, while in many regions of Europe, this practice is either prohibited or frowned upon [1]. Across North America, feeding practices range widely by region; Permittances range from allowed to strongly discouraged to illegal. In continental Europe, supplementary seasonal feeding of deer is a common practice associated with, among other things, maintaining high densities and decreasing damage to agriculture and forestry assets [11,14]. However, the “*Revier*” structure maintains these concentrations locally and in rural areas. A major difference between white-tailed deer and red deer regarding SARS-CoV-2 spillback from humans is that the former thrives seasonally at high densities in urban and peri-urban landscapes [15].

A combination of extensive versus limited feeding, which could expose animals to human exudates (including aerosols) in contaminated feed, and high density in urban and peri-urban landscapes that could expose herds to human waste, may be the key difference in SARS-CoV-2 spillback to deer populations. The lack of urban or peri-urban populations and the *Revier* management system likely limits contact among central European deer populations and may diminish human–wildlife interactions sufficiently to prevent the spread of infection. In this context, it would be advisable in Europe to maintain and strengthen barriers to spread, for example, to ensure that deer kept in wildlife parks have no contact with free-ranging deer, and to prevent deer from settling near towns and villages to minimize future spillovers. In North America, it appears important to address behaviors related to feeding of deer in urban settings and to revisit feeding practices in deer management and hunting. Maintaining natural barriers will help prevent future spillovers and spillback of SARS-CoV-2 between deer and human populations [16]. The consequences of inaction could be further spillback to humans of viral variants evolving in novel hosts, as has occurred in Danish and Dutch mink farms during the pandemic [4,5].

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microorganisms10040748/s1>, Figure S1: Full multiple sequence alignment of the ACE2 protein for the species characterized in [7] and additional deer species included from GenBank; Table S1: Sample metadata.

**Author Contributions:** Conceptualization, A.D.G. and J.F.D.; methodology, A.M.-S.; formal analysis, A.M.-S., P.D.Y.R. and G.K.M.; resources, C.W., G.Á.C., M.H.R., S.F.M. and A.P.; writing, all authors. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Regarding the ethical approval of the study, we state that all samples were taken exclusively from dead wild animals hunted by authorized Austrian and German hunters as part of game management efforts. No animals were harmed or killed specifically for the sampling, which is why German and Austrian law does not require permission from an external ethics committee in this case. However, the study was approved internally by the Internal Committee for Ethics and Animal Welfare of the Leibniz Institute for Zoo and Wildlife Research (permit #2021-09-01) and the Institutional Review Board of the German Federal Institute for Risk Assessment (project identification number BfR-SiN-08-8-002). All experiments were carried out in accordance with the approved guidelines of the IZW and Charité and comply with the laws of Germany and Austria.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** The authors thank the help of hunters and colleagues involved in the collection of the archived and recent samples. The authors wish to thank Karin Hönig and Katja Pohle for their laboratory support. We thank the German Institute for Federal Real Estate (BImA), and the German Federal Institute for Risk Assessment (BfR)-Center for Land Use Related Evaluation Methods, One Health Approaches for providing assistance and support for this study.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

- Hale, V.L.; Dennis, P.M.; McBride, D.S.; Nolting, J.M.; Madden, C.; Huey, D.; Ehrlich, M.; Grieser, J.; Winston, J.; Lombardi, D.; et al. SARS-CoV-2 Infection in Free-Ranging White-Tailed Deer. *Nature* **2021**, *602*, 481–486. [\[CrossRef\]](#) [\[PubMed\]](#)
- Chandler, J.C.; Bevins, S.N.; Ellis, J.W.; Linder, T.J.; Tell, R.M.; Jenkins-Moore, M.; Root, J.J.; Lenocho, J.B.; Robbe-Austerman, S.; DeLiberto, T.J.; et al. SARS-CoV-2 Exposure in Wild White-Tailed Deer (*Odocoileus virginianus*). *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2114828118. [\[CrossRef\]](#) [\[PubMed\]](#)
- Kuchipudi, S.V.; Surendran-Nair, M.; Ruden, R.M.; Yon, M.; Nissly, R.H.; Vandegrift, K.J.; Nelli, R.K.; Li, L.; Jayarao, B.M.; Maranas, C.D.; et al. Multiple Spillovers from Humans and Onward Transmission of SARS-CoV-2 in White-Tailed Deer. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2121644119. [\[CrossRef\]](#)
- Oreshkova, N.; Molenaar, R.J.; Vreman, S.; Harders, F.; Oude Munnink, B.B.; Hakze-van der Honing, R.W. SARS-CoV-2 Infection in Farmed Minks, The Netherlands, 2020. *Eurosurveillance* **2020**, *25*, 2001005. [\[CrossRef\]](#)
- Oude Munnink, B.B.; Sikkema, R.S.; Nieuwenhuijse, D.F.; Molenaar, R.J.; Munger, E.; Molenkamp, R. Transmission of SARS-CoV-2 on Mink Farms between Humans and Mink and Back to Humans. *Science* **2021**, *371*, 172–179. [\[CrossRef\]](#) [\[PubMed\]](#)
- Jo, W.K.; Ferreira de Oliveira-Filho, E.; Rasche, A.; Greenwood, A.D.; Osterrieder, K.; Drexler, J.F. Potential Zoonotic Sources of SARS-CoV-2 Infections. *Transbound. Emerg. Dis.* **2021**, *68*, 1824–1834. [\[CrossRef\]](#) [\[PubMed\]](#)
- Damas, J.; Hughes, G.M.; Keough, K.C.; Painter, C.A.; Persky, N.S.; Corbo, M.; Hiller, M.; Koepfli, K.-P.; Pfenning, A.R.; Zhao, H.; et al. Broad Host Range of SARS-CoV-2 Predicted by Comparative and Structural Analysis of ACE2 in Vertebrates. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 22311–22322. [\[CrossRef\]](#) [\[PubMed\]](#)
- Tan, C.W.; Chia, W.N.; Qin, X.; Liu, P.; Chen, M.I.; Tiu, C. A SARS-CoV-2 Surrogate Virus Neutralization Test Based on Antibody-Mediated Blockage of ACE2-Spike Protein-Protein Interaction. *Nat. Biotechnol.* **2020**, *38*, 1073–1081. [\[CrossRef\]](#) [\[PubMed\]](#)
- Perera, R.A.P.M.; Ko, R.; Tsang, O.T.Y.; Hui, D.S.C.; Kwan, M.Y.M.; Brackman, C.J. Evaluation of a SARS-CoV-2 Surrogate Virus Neutralization Test for Detection of Antibody in Human, Canine, Cat, and Hamster Sera. *J. Clin. Microbiol.* **2021**, *59*, e02504-20. [\[CrossRef\]](#) [\[PubMed\]](#)
- Oliveira-Filho, E.F.; Carvalho, O.V.; Carneiro, I.O.; D’ambrosio Fernandes, F.; Vaz, S.N.; Pedrosa, C.; Gonzalez-Auza, L.; Urbieto, V.C.; Kühne, A.; Mayoral, R.; et al. Frequent Infection of Cats with SARS-CoV-2 Irrespective of Pre-Existing Enzootic Coronavirus Immunity, Brazil 2020. *Front. Immunol.* **2022**. [\[CrossRef\]](#)
- Putman, R. A review of the various legal and administrative systems governing management of large herbivores in Europe. In *Ungulate Management in Europe: Problems and Practices*; Putman, R., Apollonio, R., Andersen, R., Eds.; Cambridge University Press: Cambridge, UK, 2011; pp. 54–79.



12. Organ, J.F.; Geist, V.; Mahoney, S.P.; Williams, S.; Krausman, P.R.; Batcheller, G.R.; Decker, T.A.; Charmichael, R.; Nanjappa, P.; Regan, R.; et al. The North American Model of Wildlife Conservation. In *The Wildlife Society Technical Review 12-04*; The Wildlife Society: Bethesda, MA, USA, 2012.
13. Kahlen, J.; Svadlenak-Gomez, K.; Walzer, C. ALPBIONET 2030: Wildlife Management within the EUSALP Perimeter. 2019. Available online: [https://www.alpine-space.org/projects/alpbionet2030/wpt2/d.t2.1.1-d.t2.3.1\\_wildlife-management-within-the-eusalp-perimeter.pdf](https://www.alpine-space.org/projects/alpbionet2030/wpt2/d.t2.1.1-d.t2.3.1_wildlife-management-within-the-eusalp-perimeter.pdf) (accessed on 28 March 2022).
14. Milner, J.M.; Van Beest, F.M.; Schmidt, K.T.; Brook, R.K.; Storaas, T. To Feed or Not to Feed? Evidence of the Intended and Unintended Effects of Feeding Wild Ungulates. *J. Wildl. Manag.* **2014**, *78*, 1322–1334. [[CrossRef](#)]
15. Grund, M.; McAninch, J.; Wiggers, E. Seasonal Movements and Habitat Use of Female White-Tailed Deer Associated with an Urban Park. *J. Wildl. Manag.* **2002**, *66*, 123–130. [[CrossRef](#)]
16. Reaser, J.K.; Hunt, B.E.; Ruiz-Aravena, M.; Tabor, G.M.; Patz, J.A.; Becker, D.J.; Locke, H.; Hudson, P.J.; Plowright, R.K. Fostering Landscape Immunity to Protect Human Health: A Science-Based Rationale for Shifting Conservation Policy Paradigms. *Conserv. Lett.* **2022**. [[CrossRef](#)]