



Wild Red Deer (*Cervus elaphus*) Do Not Play a Role as Vectors or Reservoirs of SARS-CoV-2 in North-Eastern Poland

Martyna Krupińska, Jakub Borkowski, Aleksander Goll, Joanna Nowicka, Karolina Baranowicz, Vincent Bourret, Tomas Strandin, Sanna Mäki, Ravi Kant, Tarja Sironen, et al.

► To cite this version:

Martyna Krupińska, Jakub Borkowski, Aleksander Goll, Joanna Nowicka, Karolina Baranowicz, et al.. Wild Red Deer (*Cervus elaphus*) Do Not Play a Role as Vectors or Reservoirs of SARS-CoV-2 in North-Eastern Poland. *Viruses*, 2022, 14 (10), pp.2290. 10.3390/v14102290 . hal-03836378

HAL Id: hal-03836378

<https://hal.inrae.fr/hal-03836378>

Submitted on 2 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.









L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Communication

Wild Red Deer (*Cervus elaphus*) Do Not Play a Role as Vectors or Reservoirs of SARS-CoV-2 in North-Eastern Poland

Martyna Krupińska¹, Jakub Borkowski² , Aleksander Goll¹, Joanna Nowicka¹ , Karolina Baranowicz¹ , Vincent Bourret^{3,4,5} , Tomas Strandin³ , Sanna Mäki³, Ravi Kant^{3,4} , Tarja Sironen^{3,4}  and Maciej Grzybek^{1,*} 

¹ Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdansk, 81-519 Gdynia, Poland

² Department of Forestry and Forest Ecology, University of Warmia and Mazury, 10-727 Olsztyn, Poland

³ Department of Virology, Medicum, University of Helsinki, 00290 Helsinki, Finland

⁴ Department of Basic Veterinary Sciences, Faculty of Veterinary Medicine, University of Helsinki, 00790 Helsinki, Finland

⁵ INRAE-Université de Toulouse UR 0035 CEFS, 31326 Castanet Tolosan, France

* Correspondence: maciej.grzybek@gumed.edu.pl; Tel.: +48-583-491-941

Abstract: Several studies reported a high prevalence of SARS-CoV-2 among white-tailed deer in North America. Monitoring cervids in all regions to better understand SARS-CoV-2 infection and circulation in other deer populations has been urged. To evaluate deer exposure and/or infection to/by SARS-CoV-2 in Poland, we sampled 90 red deer shot by hunters in five hunting districts in north-eastern Poland. Serum and nasopharyngeal swabs were collected, and then an immunofluorescent assay (IFA) to detect anti-SARS-CoV-2 antibodies was performed as well as real-time PCR with reverse transcription for direct virus detection. No positive samples were detected. There is no evidence of spillover of SARS-CoV-2 from the human to deer population in Poland.

Keywords: deer; SARS-CoV-2; cervid; susceptibility; transmission; spillover



Citation: Krupińska, M.; Borkowski, J.; Goll, A.; Nowicka, J.; Baranowicz, K.; Bourret, V.; Strandin, T.; Mäki, S.; Kant, R.; Sironen, T.; et al. Wild Red Deer (*Cervus elaphus*) Do Not Play a Role as Vectors or Reservoirs of SARS-CoV-2 in North-Eastern Poland. *Viruses* **2022**, *14*, 2290. <https://doi.org/10.3390/v14102290>

Academic Editor: Subir Sarker

Received: 1 October 2022

Accepted: 17 October 2022

Published: 18 October 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/>).

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the COVID-19 pandemic, can infect multiple wild and domestic animals [1–6]. Due to the possible maintenance, dissemination and evolution of the virus, there is an urgent need to identify and monitor species susceptible to SARS-CoV-2. Animal reservoirs constitute a threat by contributing to animal–animal and animal–human transmission of viruses. Such transmissions can lead to reverse zoonosis (spillover) of novel animal-adapted variants that the human immune system has not encountered before [7,8]. This phenomenon is well documented for SARS-CoV-2 infections of farmed mink (*Neovison vison*). After the first detection of the virus in mink in the Netherlands [4], extensive surveillance of mink farms has been implemented, and SARS-CoV-2-infected mink have been detected worldwide [9,10]. Additionally, in the Netherlands, Denmark and Poland, SARS-CoV-2 strains with an animal sequence signature were detected among farm employees and other contact individuals, indicating spillover from mink to humans [11–14]. Likewise, the circulation of SARS-CoV-2 among free-roaming wild animals poses challenges for surveillance and control, and abundant animals living close to urban settlements should be paid the utmost attention [8].

In July 2021, antibodies against SARS-CoV-2 were detected among 40% (152/624 samples) of studied white-tailed deer (*Odocoileus virginianus*) during wildlife disease surveillance operations in the United States [15]. Previous studies had shown that white-tailed deer fawns, when experimentally inoculated with SARS-CoV-2, shed infective virus up to 5 days after infection and developed antibodies against the virus [16]. Vertical and

horizontal virus transmission has also been documented [17]. In September 2021, Hale et al. employed the real-time PCR method and detected SARS-CoV-2 in 35.8% of 360 free-range white-tailed deer [18]. Moreover, white-tailed deer have been reported to have been exposed to different SARS-CoV-2 variants during separate events [18,19]. Further serological and molecular studies have also investigated the role of white-tailed deer as a reservoir of SARS-CoV-2 [20–23], and the first suspected deer-to-human transmission has been reported recently [24].

In response to the high numbers of infected deer in North America, the World Organisation for Animal Health (WOAH) recommended monitoring cervids in all regions to better understand SARS-CoV-2 infection and circulation in other deer populations [25]. Recent European efforts have sought to determine whether other deer species can also act as reservoirs for the SARS-CoV-2 virus. Serological studies conducted in Germany, Austria and the United Kingdom found no evidence of spillover of SARS-CoV-2 to cervid species in these regions [26–28].

The red deer (*Cervus elaphus*) is one of the most widespread large mammals in Europe [29]. According to the Central Statistical Office in Poland, the national population of red deer increased significantly from 180 thousand to 281.9 thousand over the last eleven years, increasing the risk of exposure to deer in urban environments [30]. Furthermore, an in silico study of ACE2 (angiotensin-I-converting enzyme 2) receptors demonstrated that ACE2 from all three studied Cervidae species—the white-tailed deer, reindeer (*Rangifer tarandus*) and Père David’s deer (*Elaphurus davidianus*)—are at high risk of binding SARS-CoV-2 receptor-binding domain [29]. Apart from K-N substitution, no differences have been found between the red deer and white-tailed deer ACE2 amino acid sequence [28].

As a means for proactive, targeted monitoring of wildlife, we investigated whether a spillover of SARS-CoV-2 occurred among red deer in Poland.

2. Materials and Methods

2.1. Material Collection and RNA Isolation

Samples were collected from 90 red deer (*Cervus elaphus*) individuals shot by hunters in Warminsko-Mazurskie Voivodeship in north-eastern Poland (Figure 1).

Samples were collected in Strzalow, Olsztyn, Milomlyn, Nidzica and Nowe Ramuki. Table 1 shows the number and status of sampled individuals.

Table 1. Location, status, and number of red deer (*Cervus elaphus*) individuals sampled.

Hunting District/Deer	Doe	Bull	Fawn	Total
Strzalow	16	2	0	18
Olsztyn	12	4	3	19
Milomlyn	4	0	1	5
Nidzica	8	4	4	16
Nowe Ramuki	20	7	5	32
Total	60	17	13	90

Blood or blood clots were collected and centrifuged to obtain serum. Since animal carcasses were stiff, we used a sterile plastic speculum to open the nasal cavity; then, using a thick swab, we collected nasopharyngeal swabs and preserved them in a virus deactivation buffer at +4 °C. A total of 150 µL from each sample of a swab in inactivation buffer was added to 300 µL of RLT lysis buffer (RNeasy Mini kit, Qiagen, Germany). Samples were mixed by vortexing and incubated for 10 min at room temperature. After incubation, 400 µL of 70% ethanol was added to each sample and mixed by pipetting. The lysate was transferred to a RNeasy Mini spin column with a collection tube and centrifuged for 1 min at 13,000 RPM. Columns were washed once with 700 µL RW1 and twice with 500 µL RPE. Between every wash, the columns were centrifuged and the flow-through was discarded.

Elution was performed by adding 50 μ L of PCR-grade water to the column and incubating for 2 min. Columns were placed into new tubes and centrifuged at 13,000 RPM for 1 min. After isolation, the samples were processed. We tested 90 nasopharyngeal swab samples by RT-rtPCR and 90 serum samples by immunofluorescent assay (IFA). No human-origin samples were processed at the same time in the laboratory.

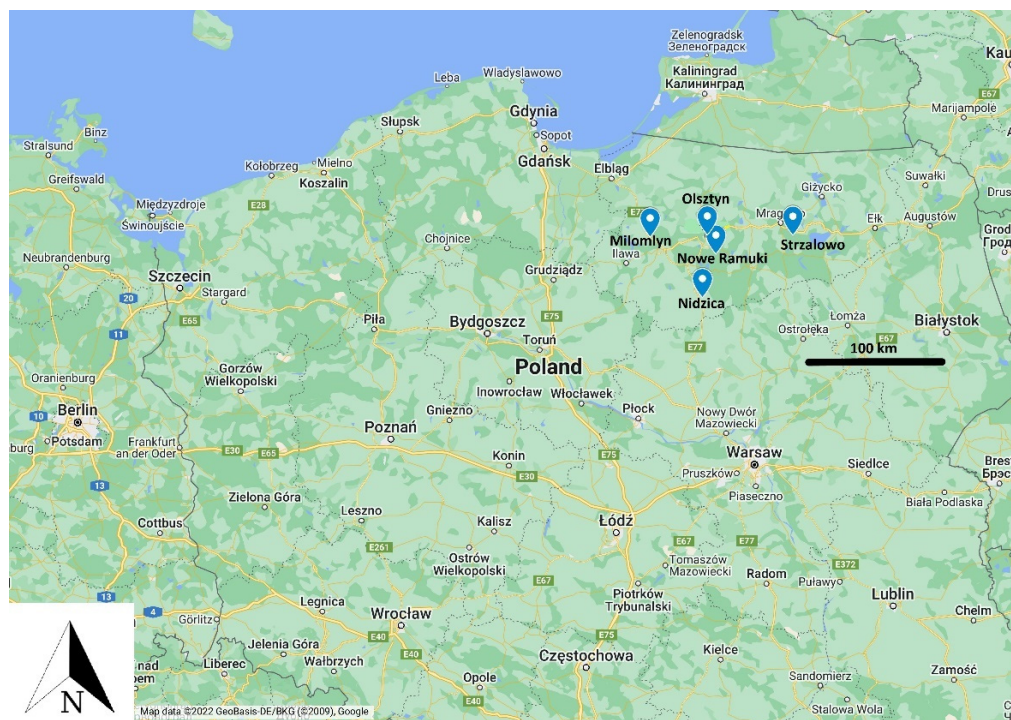


Figure 1. Four hunting districts are located in north-eastern Poland. The black bar indicates 100 km distance (Map data, Google, 2022).

2.2. SARS-CoV-2 Case Definition

A SARS-CoV-2-positive individual was defined as suggested by WOA [31]. Deer are considered SARS-CoV-2 positive if: SARS-CoV-2 has been isolated from a sample taken directly from an animal (nasal swab, oropharyngeal swab) or viral nucleic acid has been identified in a sample taken directly from an animal, giving cause for the suspicion of a previous association or contact with SARS-CoV-2 by (a) targeting at least two specific genomic regions at a level indicating the presence of infectious virus or (b) targeting a single genomic region followed by sequencing of a secondary target.

2.3. Real-Time RT-PCR

For each sample, the reaction mixture was prepared using a TaqPath™ 1-Step RT-qPCR Master Mix (ThermoFisher Scientific, Waltham, MA, USA), polymerase, DEPC-treated water (EURx, Gdańsk, Poland), primers and probes for the RNA-dependent RNA polymerase (RdRp) and envelope (E) genes [31] in white 8-well qPCR strips with optical clear caps. Positive control plasmids were prepared in-house with the RdRp and E genes and a no-template control (NTC) containing DEPC-treated water instead of template reactions. Reactions were mixed and loaded into a Light Cycler 480 (Applied Biosystems, Waltham, USA). Cycling conditions were Uracil N-glycosylase (UNG) incubation for 2 min at 25 °C, RT incubation for 15 min at 50 °C, and enzyme activation for 2 minutes at 95 °C, followed by 40 amplification cycles consisting of 3 seconds at 95 °C, and 30 s at 60 °C. After each amplification cycle, the signal from each sample was measured in both the FAM (RdRp gene) and HEX (E gene) channels. Samples with $C_p < 35$ for either gene were considered positive for SARS-CoV-2.

2.4. Immunofluorescent Assay (IFA) for Antibodies against SARS-CoV-2 Detection

Deer serum samples were analysed using an immunofluorescence assay (IFA) with seropositive human serum as a positive control as previously described [3,32–34]. Serum samples were diluted 1:10 in PBS, and the reactivity of the samples to SARS-CoV-2 was tested with SARS-CoV-2-IFA. Infected Vero E6 cells were detached with trypsin, mixed with uninfected Vero E6 cells (in a ratio of 1:3), washed with PBS, spotted on IFA slides, air-dried, and fixed with acetone. The slides were stored at -70°C until use. We used rabbit anti-deer IgG fluorescein isothiocyanate labeled as a conjugate (LGC Sera Care, Milford, CT, USA). The slides were read under a fluorescence microscope, and pictures were taken with a ZOETM fluorescent cell imager (BioRad, Hercules, USA).

3. Results

Real-time RT-PCR and IFA approach failed to detect any SARS-CoV-2 positive samples among the 90 assayed. The estimated prevalence and seroprevalence for SARS-CoV-2 in the investigated red deer population was 0% [0.0–6.7].

4. Discussion

We did not detect either the SARS-CoV-2 virus or antibodies against the virus in wild red deer in Poland. There is no evidence that spillover of SARS-CoV-2 from human to deer populations occurred in the studied territory. Our results align with other European countries' reports on wild deer populations [26–28]. Comprehensive studies from the United Kingdom [26], Austria and Germany [28] showed no sign of SARS-CoV-2 in several deer species. This result supports the hypothesis that wild deer are not currently a reservoir for SARS-CoV-2 in Europe.

In contrast to European results, the high prevalence and seroprevalence of SARS-CoV-2 in white-tailed deer in North America have been reported in several studies [15,18]. Multiple spillovers of SARS-CoV-2 from humans to white-tailed deer have been documented, along with deer-to-deer transmission. A recent report by Pickering et al. [24] found an epidemiologically linked human case indicating spillback from deer to humans.

There are several factors to consider to explain observed differences in infection rates in deer populations from North America and Europe. The high prevalence in white-tailed deer may be explained by its ACE2 receptor specificity. In silico modelling studies suggest that other deer species might also be susceptible to SARS-CoV-2 [28,35]. Although K-N substitution between red deer and white-tailed deer ACE2 sequence occurs, it fails to explain the lack of detected infections in other European deer species with an ACE2 receptor sequence identical to white-tailed deer. Crucial factors to consider are the deer population distribution, ecology, and behavioural differences. White-tailed deer are often reported to inhabit urban and peri-urban environments [36–38].

In contrast, red deer found in European forests are rarely reported to visit human settlements [39]. They are considered to be timid and avoid contact with humans. This may explain our and other groups' results, where red deer were found to be negative for SARS-CoV-2 screening [26–28]. Although routes of white-tailed deer infection are unclear, deer social behaviour might be the factor facilitating the quick spread of the pathogen. Female white-tailed deer live in small herds, while males have broader territories and social contacts, increasing the risk of contact with the virus [40]. Intermediate hosts, such as mink, enabling transmission also cannot be ruled out [24]. Differences in hunting practices should also be considered; for example, animal baiting is allowed in over 20 out of 50 states in North America, which may cause indirect contact with humans, while this practice is forbidden in Poland, as in many other European countries [28].

This study suggests that common wild European red deer are not currently supporting SARS-CoV-2 infections. However, considering reports from North America, it is necessary to monitor wildlife for spillover and spillbacks. Biomonitoring is one of the most effective methods of predicting and preventing possible epidemics [33,41–44]. Searching for novel hosts or reservoirs of zoonotic pathogens should be a priority for public health and wildlife

management institutions [32,45]. The introduction of SARS-CoV-2 to wildlife has caused the establishment of animal reservoirs (i.e., white-tail deer). Therefore, all efforts should be made to reduce the risk of new variant emergence and to protect both humans and wildlife (FAO, WHO, WOA, 2021 [46]). We believe that preparation for a “Pathogen X” pandemic should employ a “One Health” approach with a strong emphasis on monitoring both domestic and wild animals.

Author Contributions: Conceptualisation, M.G. and J.B.; methodology, R.K., T.S. (Tomas Strandin), J.B. and M.G.; investigation, M.K., A.G., K.B., T.S. (Tarja Sironen), S.M., J.N. and M.G.; resources, M.G.; data curation, S.M. and M.G.; writing, original draft preparation, M.K., M.G. and R.K.; writing—review and editing, M.G., V.B. and T.S. (Tarja Sironen); supervision, M.G. and T.S. (Tarja Sironen); project administration, M.G. and J.B.; funding acquisition, M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was co-funded through the 2018–2019 BiodivERsA joint call for research proposals under the BiodivERsA3 ERA-Net COFUND programme; the funding organisations ANR (France), DFG (Germany), EPA (Ireland), FWO (Belgium), and NCN (Poland). M.G., J.N. and A.G. were supported by the National Science Centre, Poland, under the BiodivERsA3 programme (2019/31/Z/NZ8/04028). M.K. was supported by the National Science Centre, Poland, under the Preludium BIS programme 2020/39/O/NZ6/01777.

Institutional Review Board Statement: Ethical review and approval were waived for this study since biological material came from dead animals shot by hunters according to Polish national law on hunting (Polish Hunting Law-Act Dz. U. 1995 Nr 147 poz. 713 published on 13 October 1995).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available from authors upon request.

Acknowledgments: We thank the local hunter societies for helping us in sample collection; Alicja Rost and Ewa Zielińiewicz for laboratory assistance; and the University of Gdańsk, Medical University of Gdańsk, and University of Helsinki for their support.

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

References

1. Shan, C.; Yao, Y.F.; Yang, X.L.; Zhou, Y.W.; Gao, G.; Peng, Y.; Yang, L.; Hu, X.; Xiong, J.; Jiang, R.D.; et al. Infection with novel coronavirus (SARS-CoV-2) causes pneumonia in Rhesus macaques. *Cell Res.* **2020**, *30*, 670. [[CrossRef](#)] [[PubMed](#)]
2. Shi, J.; Wen, Z.; Zhong, G.; Yang, H.; Wang, C.; Huang, B.; Liu, R.; He, X.; Shuai, L.; Sun, Z.; et al. Susceptibility of ferrets, cats, dogs and other domesticated animals to SARS-coronavirus 2. *Science* **2020**, *368*, 1016–1020. [[CrossRef](#)]
3. Rabalski, L.; Kosinski, M.; Smura, T.; Aaltonen, K.; Kant, R.; Sironen, T.; Szewczyk, B.; Grzybek, M. Severe Acute Respiratory Syndrome Coronavirus 2 in Farmed Mink (*Neovison vison*), Poland. *Emerg. Infect. Dis.* **2021**, *27*, 2333–2339. [[CrossRef](#)]
4. Oreshkova, N.; Molenaar, R.J.; Vreman, S.; Harders, F.; Oude Munnink, B.B.; Hakze-van der Honing, R.W.; Gerhards, N.; Tolsma, P.; Bouwstra, R.; Sikkema, R.S.; et al. SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. *Eurosurveillance* **2020**, *25*, 2001005. [[CrossRef](#)]
5. Mahdy, M.A.A.; Younis, W.; Ewaida, Z. An Overview of SARS-CoV-2 and Animal Infection. *Front. Vet. Sci.* **2020**, *7*, 596391. [[CrossRef](#)]
6. Rodriguez-Morales, A.J.; Dhama, K.; Sharun, K.; Tiwari, R.; Bonilla-Aldana, D.K. Susceptibility of felids to coronaviruses. *Vet. Rec.* **2020**, *186*, e21. [[CrossRef](#)] [[PubMed](#)]
7. Banerjee, A.; Mossman, K.; Baker, M.L. Zoonotic potential of SARS-CoV-2 and implications of reintroduction into human populations. *Cell Host Microbe* **2021**, *29*, 160–164. [[CrossRef](#)] [[PubMed](#)]
8. Delahay, R.J.; de la Fuente, J.; Smith, G.C.; Sharun, K.; Snary, E.L.; Flores Girón, L.; Nziza, J.; Fooks, A.R.; Brookes, S.M.; Lean, F.Z.X.; et al. Assessing the risks of SARS-CoV-2 in wildlife. *One Heal. Outlook* **2021**, *3*, 7. [[CrossRef](#)] [[PubMed](#)]
9. Koopmans, M. SARS-CoV-2 and the human-animal interface: Outbreaks on mink farms. *Lancet Infect. Dis.* **2020**, *21*, 18–19. [[CrossRef](#)]
10. Farag, E.A.; Islam, M.M.; Enan, K.; El-Hussein, A.-R.M.; Bansal, D.; Haroun, M. SARS-CoV-2 at the human-animal interface: A review. *Heliyon* **2021**, *7*, e08496. [[CrossRef](#)]
11. Oude Munnink, B.B.; Sikkema, R.S.; Nieuwenhuijse, D.F.; Molenaar, R.J.; Munger, E.; Molenkamp, R.; van der Spek, A.; Tolsma, P.; Rietveld, A.; Brouwer, M.; et al. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science* **2020**, *371*, eabe5901. [[CrossRef](#)]

12. Boklund, A.; Hammer, A.S.; Quaade, M.L.; Rasmussen, T.B.; Lohse, L.; Strandbygaard, B.; Jørgensen, C.S.; Olesen, A.S.; Hjerpe, F.B.; Petersen, H.H.; et al. SARS-CoV-2 in Danish Mink Farms: Course of the Epidemic and a Descriptive Analysis of the Outbreaks in 2020. *Animals* **2021**, *11*, 164. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Rabalski, L.; Kosinski, M.; Mazur-Panasiuk, N.; Szewczyk, B.; Bienkowska-Szewczyk, K.; Kant, R.; Sironen, T.; Pyrc, K.; Grzybek, M. Zoonotic spill-over of SARS-CoV-2: Mink-Adapted virus in humans. *Clin. Microbiol. Infect.* **2022**, *28*, 451.e1–451.e4. [\[CrossRef\]](#)
14. Hammer, A.S.; Quaade, M.L.; Rasmussen, T.B.; Fonager, J.; Rasmussen, M.; Mundbjerg, K.; Lohse, L.; Strandbygaard, B.; Jørgensen, C.S.; Alfaro-Núñez, A.; et al. SARS-CoV-2 Transmission between Mink (*Neovison vison*) and Humans, Denmark. *Emerg. Infect. Dis.* **2021**, *27*, 547. [\[CrossRef\]](#)
15. 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. [\[CrossRef\]](#)
16. Palmer, M.V.; Martins, M.; Falkenberg, S.; Buckley, A.; Caserta, L.C.; Mitchell, P.K.; Cassmann, E.D.; Rollins, A.; Zylich, N.C.; Renshaw, R.W.; et al. Susceptibility of White-Tailed Deer (*Odocoileus virginianus*) to SARS-CoV-2. *J. Virol.* **2021**, *95*, e00083-21. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Cool, K.; Gaudreault, N.N.; Morozov, I.; Trujillo, J.D.; Meekins, D.A.; McDowell, C.; Carossino, M.; Bold, D.; Mitzel, D.; Kwon, T.; et al. Infection and transmission of ancestral SARS-CoV-2 and its alpha variant in pregnant white-tailed deer. *Emerg. Microbes Infect.* **2022**, *11*, 95–112. [\[CrossRef\]](#)
18. 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* **2022**, *602*, 481–486. [\[CrossRef\]](#)
19. Vandegrift, K.J.; Yon, M.; Surendran-Nair, M.; Gontu, A.; Amirthalingam, S.; Nissly, R.H.; Levine, N.; Stuber, T.; DeNicola, A.J.; Boulanger, J.R.; et al. Detection of SARS-CoV-2 Omicron variant (B.1.1.529) infection of white-tailed deer. *bioRxiv Prepr. Serv. Biol.* **2022**.
20. Roundy, C.M.; Nunez, C.M.; Thomas, L.F.; Auckland, L.D.; Tang, W.; Richison, J.J.; Green, B.R.; Hilton, C.D.; Cherry, M.J.; Pauvolid-Corrêa, A.; et al. High Seroprevalence of SARS-CoV-2 in White-Tailed Deer (*Odocoileus virginianus*) at One of Three Captive Cervid Facilities in Texas. *Microbiol. Spectr.* **2022**, *10*, e00576-22. [\[CrossRef\]](#)
21. 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. [\[CrossRef\]](#)
22. Kotwa, J.D.; Massé, A.; Gagnier, M.; Aftanas, P.; Blais-Savoie, J.; Bowman, J.; Buchanan, T.; Chee, H.-Y.; Dibernardo, A.; Kruczkiewicz, P.; et al. First detection of SARS-CoV-2 infection in Canadian wildlife identified in free-ranging white-tailed deer (*Odocoileus virginianus*) from southern Québec, Canada. *bioRxiv* **2022**, 476458.
23. Palermo, P.M.; Orbegozo, J.; Watts, D.M.; Morrill, J.C. SARS-CoV-2 Neutralizing Antibodies in White-Tailed Deer from Texas. *Vector-Borne Zoonotic Dis.* **2021**, *22*, 62–64. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Pickering, B.; Lung, O.; Maguire, F.; Kruczkiewicz, P.; Kotwa, J.D.; Buchanan, T.; Gagnier, M.; Guthrie, J.L.; Jardine, C.M.; Marchand-Austin, A.; et al. Highly divergent white-tailed deer SARS-CoV-2 with potential deer-to-human transmission. *bioRxiv* **2022**, 481551.
25. World Organisation for Animal Health OIE Statement on Monitoring White-Tailed Deer for SARS-CoV-2; World Organisation for Animal Health: Paris, France, 2022.
26. Holding, M.; Otter, A.D.; Dowall, S.; Takumi, K.; Hicks, B.; Coleman, T.; Hemingway, G.; Royds, M.; Findlay-Wilson, S.; Curran-French, M.; et al. Screening of wild deer populations for exposure to SARS-CoV-2 in the United Kingdom, 2020–2021. *Transbound. Emerg. Dis.* **2022**, *69*, e3244–e3249. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Wernike, K.; Fischer, L.; Holsteg, M.; Aebischer, A.; Petrov, A.; Marquart, K.; Schotte, U.; Schön, J.; Hoffmann, D.; Hechinger, S.; et al. Serological screening in wild ruminants in Germany, 2021/2022: No evidence of SARS-CoV-2, bluetongue virus or pestivirus spread but high seroprevalences against Schmallenberg virus. *Transbound. Emerg. Dis.* **2022**, *69*, e3289–e3296. [\[CrossRef\]](#)
28. 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. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Zachos, F.E.; Hartl, G.B. Phylogeography, population genetics and conservation of the European red deer *Cervus elaphus*. *Mamm. Rev.* **2011**, *41*, 138–150. [\[CrossRef\]](#)
30. Główny Urząd Statystyczny. Obszary Tematyczne/Roczniki Statystyczne/Roczniki Statystyczne/Rocznik Demograficzny. Główny Urząd Statystyczny: Warsaw, Poland, 2013.
31. World Organisation for Animal Health Infection with SARS-COV-2 in Animals; World Organisation for Animal Health: Paris, France, 2022.
32. Grzybek, M.; Sironen, T.; Mäki, S.; Tołkacz, K.; Alsarra, M.; Strachecka, A.; Paleolog, J.; Biernat, B.; Szczepaniak, K.; Behnke-Borowczyk, J.; et al. Zoonotic Virus Seroprevalence among Bank Voles, Poland, 2002–2010. *Emerg. Infect. Dis.* **2019**, *25*, 1607–1609. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Bourret, V.; Dutra, L.; Alburkat, H.; Mäki, S.; Lintunen, E.; Wasniewski, M.; Kant, R.; Grzybek, M.; Venkat, V.; Asad, H.; et al. Serological surveillance for wild rodent infection with SARS-CoV-2 in Europe. *bioRxiv* **2022**, 502439.

34. Grzybek, M.; Tołkacz, K.; Sironen, T.; Mäki, S.; Alsarraf, M.; Behnke-Borowczyk, J.; Biernat, B.; Nowicka, J.; Vaheri, A.; Henttonen, H.; et al. Zoonotic Viruses in Three Species of Voles from Poland. *Animals* **2020**, *10*, 1820. [[CrossRef](#)] [[PubMed](#)]
35. 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](#)]
36. Grund, M.D.; McAninch, J.B.; Wiggers, E.P. Seasonal Movements and Habitat Use of Female White-Tailed Deer Associated with an Urban Park. *J. Wildl. Manage.* **2002**, *66*, 123. [[CrossRef](#)]
37. Blanchong, J.A.; Sorin, A.B.; Scribner, K.T. Genetic diversity and population structure in urban white-tailed deer. *J. Wildl. Manage.* **2013**, *77*, 855–862. [[CrossRef](#)]
38. Etter, D.R.; Hollis, K.M.; Van Deelen, T.R.; Ludwig, D.R.; Chelsvig, J.E.; Anchor, C.L.; Warner, R.E. Survival and Movements of White-Tailed Deer in Suburban Chicago, Illinois. *J. Wildl. Manage.* **2002**, *66*, 500. [[CrossRef](#)]
39. Coppes, J.; Burghardt, F.; Hagen, R.; Suchant, R.; Braunisch, V. Human recreation affects spatio-temporal habitat use patterns in red deer (*Cervus elaphus*). *PLoS ONE* **2017**, *12*, e0175134. [[CrossRef](#)]
40. Gear, D.A.; Samuel, M.D.; Scribner, K.T.; Weckworth, B.V.; Langenberg, J.A. Influence of genetic relatedness and spatial proximity on chronic wasting disease infection among female white-tailed deer. *J. Appl. Ecol.* **2010**, *47*, 532–540. [[CrossRef](#)]
41. Walton, L.; Marion, G.; Davidson, R.S.; White, P.C.L.; Smith, L.A.; Gavier-Widen, D.; Yon, L.; Hannant, D.; Hutchings, M.R. The ecology of wildlife disease surveillance: Demographic and prevalence fluctuations undermine surveillance. *J. Appl. Ecol.* **2016**, *53*, 1460–1469. [[CrossRef](#)]
42. Grzybek, M.; Alsarraf, M.; Tołkacz, K.; Behnke-Borowczyk, J.; Biernat, B.; Stańczak, J.; Strachecka, A.; Guz, L.; Szczepaniak, K.; Paleolog, J.; et al. Seroprevalence of TBEV in bank voles from Poland—a long-term approach. *Emerg. Microbes Infect.* **2018**, *7*, 145. [[CrossRef](#)]
43. Grzybek, M.; Tołkacz, K.; Alsarraf, M.; Dwużnik, D.; Szczepaniak, K.; Tomczuk, K.; Biernat, B.; Behnke, J.M.; Bajer, A. Seroprevalence of Tick-Borne Encephalitis Virus in Three Species of Voles (*Microtus* spp.) in Poland. *J. Wildl. Dis.* **2020**, *56*, 492. [[CrossRef](#)]
44. Tołkacz, K.; Alsarraf, M.; Kowalec, M.; Dwużnik, D.; Grzybek, M.; Behnke, J.M.; Bajer, A. Bartonella infections in three species of *Microtus*: Prevalence and genetic diversity, vertical transmission and the effect of concurrent *Babesia microti* infection on its success. *Parasit. Vectors* **2018**, *11*, 491. [[CrossRef](#)]
45. Grzybek, M.; Cybulska, A.; Tołkacz, K.; Alsarraf, M.; Behnke-Borowczyk, J.; Szczepaniak, K.; Strachecka, A.; Paleolog, J.; Moskwa, B.; Behnke, J.M.; et al. Seroprevalence of *Trichinella* spp. infection in bank voles (*Myodes glareolus*)—A long term study. *Int. J. Parasitol. Parasites Wildl.* **2019**, *9*, 144–148. [[CrossRef](#)]
46. Food and Agriculture Organization, World Organisation for Animal Health, and World Health Organization. *Joint Statement on the Prioritisation of Monitoring SARS-CoV-2 Infection in Wildlife and Preventing the Formation of Animal Reservoirs*; World Organisation for Animal Health: Paris, France, 2022.