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Presence of anti-SARS-CoV-2 antibodies in European bison (*Bison bonasus*) in Poland, 2019–2023

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Abstract

Background The origin of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains unknown. However, it is likely that the virus spillover occurred from an animal reservoir to humans. Identifying animal species susceptible to SARS-CoV-2 is crucial for understanding cross-species transmission to humans. This study distinguishes itself by focusing on the susceptibility of the European bison (*Bison bonasus*), an endangered species, to SARS-CoV-2. The objective of this study was to investigate the occurrence of SARS-CoV-2 antibodies in a substantial number ($n = 238$) of both free-living and captive Polish European bison using an in-house ELISA method and virus neutralization test (VNT).

Results The seroprevalence of SARS-CoV-2 infection was found to be 1.29% (3/232). None of the seropositive European bison tested positive in the virus neutralization test. All seropositive animals were part of captive herds.

Conclusions This study represents the first report of SARS-CoV-2 seroprevalence in both free-ranging and captive European bison in Poland. Based on these findings, the European bison appears to be a less susceptible species to SARS-CoV-2. The most probable route of transmission was from humans to European bison, as all seropositive animals belonged to captive herds with contact with indirect human sources, such as tourists and keepers.

Keywords *Bison bonasus*, ELISA, Poland, SARS-Cov-2, Serology, VNT

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Background

Coronavirus disease 2019 (COVID-19) in humans is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel coronavirus belonging to the Coronaviridae family [1]. Epidemiological investigations into the initial human cases of COVID-19 have revealed their association with the Huanan seafood market in Wuhan. Consequently, there is a suggestion that SARS-CoV-2 may have originated from animal hosts and transmitted to humans [2, 3]. In light of this, numerous animal species, including wildlife, have been subjected to SARS-CoV-2 testing [4, 5].

The spike (S) glycoprotein, responsible for binding to receptor cells, plays a crucial role in determining the host range of coronaviruses [6]. In mammals, the primary receptor for SARS-CoV-2 is the angiotensin I converting enzyme 2 (ACE2), a receptor that is conserved across various animal species [7]. Despite extensive studies on the susceptibility of a broad range of mammals (252 species) to SARS-CoV-2, there is currently no data available regarding the European bison *(Bison bonasus*). However, closely related species such as the American bison (*Bison bison*) have been categorized within the medium class of susceptibility to SARS-CoV-2. This classification is based on the conservation of receptor properties and the ability of SARS-CoV-2 to bind to them [7].

The European bison, the largest herbivore in Europe, is an endangered species classified as near threatened by the International Union for Conservation of Nature (IUCN) [8]. Most European bison populations reside in free-ranging herds located in Poland, Belarus, and Russia. These European bison have been exposed to various zoonotic agents, including *Mycobacterium caprae* [9], *Truperella pyogenes* [10], and *Brucella* spp [11]., primarily through interactions with livestock [12]. Active conservation efforts for the European bison population should include comprehensive health monitoring [13].

No data currently exists regarding the prevalence of SARS-CoV-2 in European bison. Thus, this study represents the first seroepidemiological investigation of SARS-CoV-2 in a substantial number of Polish European bison. Given the status of the European bison as an endangered species, it is necessary to monitor the emergence of new pathogens, particularly in this vulnerable population.

Results

A total of three animals (3/232; 1.29%) were identified as seropositive using the in-house ELISA method, with the following identification numbers: 422, 426, and 452. All positive animals were male and resided in captive herds located in different regions: Pszczyna, Muczne in the Bieszczady Mountains, and Gołuchów. The seropositive samples were collected on various dates: November 2021 (ID 422), December 2021 (ID 426), and June 2022 (ID 452).

Each seropositive animal's habitat differed as follows: ID 422 was from the Muczne captive herd in the Bieszczady Mountains, characterized by the absence of neighbouring ungulates and European bison observable by tourists. These animals are fed hay by breeders. ID 426 was located in Gołuchów, while ID 452 was found in Pszczyna. In the cases of European bison from Gołuchów and Pszczyna, both animals were part of enclosed herds. Those herds were inhabited by neighboring ungulates. The area was accessible to tourists, resembling more of a zoo-like setting where they are also fed hay by breeders.

These locations are European bison demonstrations and enclosures open to tourists, where the animals are cared for and fed by staff. The seropositive animals displayed no clinical signs of illness and were immobilized to undergo health assessments before transportation.

The seroprevalence of SARS-CoV-2 infection was determined to be 1.29% (95% Confidence Interval (CI): 0.36–3.79). Among the seropositive European bison, optical density (OD) units ranged from 0.29 to 0.31, with a cut-off value of ≥0.29. None of the seropositive European bison tested positive for the virus neutralization test (VNT).

No significant association ($p > 0.05$) was observed between SARS-CoV-2 seropositivity as determined by ELISA and age or sex. However, a significant association was found between captivity lifestyle and SARS-CoV-2 seropositivity ($p = 0.004$).

Discussion

The current study represents the initial screening of European bison in Europe for exposure to SARS-CoV-2. Samples collected from animals included in this study originated from various regions of Poland, encompassing a significant number of European bison. The observed seroprevalence level of 1.29% indicates that *Bison bonasus* can indeed be infected with SARS-CoV-2. However, the limited number of seropositive animals suggests a low susceptibility of this species to SARS-CoV-2 infection.

In Poland, various epidemiological studies have investigated the SARS-CoV-2 seroprevalence in cats and dogs [14, 15]. One such study conducted in Poland collected samples from four different locations, namely Poznan, Przemysl, Kluczbork, and Dęblin, between June 2020 and February 2021, during the second wave of the COVID-19 outbreak. This study reported a seroprevalence of SARS-CoV-2 in cats and dogs of 1.79% and 1.17%, respectively [14]. Another epidemiological study, conducted during the fourth wave of the COVID-19 outbreak between October 2021 and February 2022 in Gdansk and Olsztyn cities, revealed a higher seroprevalence, with 18.9%

of feline samples and 16.0% of canine samples testing positive for SARS-CoV-2 antibodies [15]. The differences observed between waves of the COVID-19 outbreak in Poland reflect fluctuations in human cases, with varying impacts depending on the analyzed wave [16]. Similarly, in dogs and cats, there was a notable increase in seroprevalence during the fourth wave compared to the second wave of the COVID-19 outbreak [15].

In the present study, samples were collected spanning from the first wave to the seventh wave of the COVID-19 outbreak. Seropositive animals were detected in November 2021 ($n = 1$), December 2021 ($n = 1$), and June 2022 ($n = 1$), which coincided with the fourth and fifth waves of the COVID-19 outbreak, during which the Omicron variant was present in Poland. Despite this broad sampling period, no significant level of exposure was detected among the European bison population, suggesting a limited risk of exposure to SARS-CoV-2 for these animals.

We have found a significant association between living in captivity and SARS-CoV-2 seropositivity. This is in line with previous studies regarding other animal species. Most of the seropositive animals were detected after contact with people, some of them could be considered as apparently healthy infected status with the risk of transmission between humans and animals. These circumstances have been described in the zoological garden in felids [17].

Seropositive animals were discovered in three distinct areas; however, all these animals resided in captive herds with the potential for contact with asymptomatic infected individuals. Instances of SARS-CoV-2 transmission from humans to animals have been documented across various species, including American mink [18], as well as zoo animals like lions, tigers [19], great apes [20, 21], and non-human primates [22]. Given the close and sustained interactions with humans, particularly in open facilities where direct contact with the public is common, the potential for human-to-European bison transmission could be justified. It is noteworthy that other animals from the same herds tested negative for antibodies. Nevertheless, the dynamics of SARS-CoV-2 antibodies in European bison remain unknown. For domestic animals like dogs and cats, studies have shown the persistence of SARS-CoV-2 neutralizing antibodies up to 10 months after initial detection in dogs [23] and up to 18 months in cats [24]. In the case of wildlife species, such as white-tailed deer (*Odocoileus virginianus*), neutralizing antibodies have been observed to persist for more than 13 months in naturally infected captive animals [25].

In our study, the absence of additional seropositive animals within the same herd could be explained by the low susceptibility of European bison to SARS-CoV-2 infection. We can assume low susceptibility by taking a low number of seropositive animals during the duration of the study. Moreover, low levels of anti-SARS-CoV–2 together with the negative result in VNT, suggest that European bison are not very susceptible, similar to other ruminants such as sheep [26].

Probably there is no transmission between European bison as if there would be transmission we will predict more cases in one herd. However, in some species transmission within one animals species was confirmed, e.g. in white-tailed deer (*Odocoileus virginianus*) [27]. Cases of seropositive animals in our study may be a result of the close contact between animals and humans, potentially facilitating human-to-European bison transmission. This suggests that the lack of further seropositive cases may not be due to a rapid decline in antibody levels post-SARS-CoV-2 infection, but rather to the dynamics of transmission within the specific context of human-European bison interaction.

It is crucial to emphasize that investigating SARS-CoV-2 infections in wild animals is significant within the broader context of viral evolution. These animals have the potential to act as reservoirs, posing a future risk of reinfection in humans. Such discoveries underscore the necessity for ongoing vigilance and research to protect the health of both animals and humans amidst our evolving interaction with SARS-CoV-2. In general terms, from a passive search perspective for SARS-CoV-2 infection in wildlife, serological techniques appear to have a superior capacity for detecting virus exposure compared to molecular techniques. This is primarily due to the persistence over time of antibodies that can be detected by serological methods and the challenge of detecting clinically symptomatic animals with a brief virus-shedding period using molecular tests.

In our study, VNT did not yield positive results for SARS-CoV-2, which aligns with findings from studies conducted in ferrets [28, 29] and cats [25]. In these studies discordant results between receptor-binding domain (RBD)-based ELISA and VNT tests were observed, especially in samples with low anti-RBD IgG antibody levels. Furthermore, we did not observe neutralization in the neutralization studies conducted, which correlates with findings from a study conducted in humans. This study observed that the concentration of IgG corresponds the viral neutralizing activity of plasma and serum from convalescent patients against SARS-CoV-2. Not all antibodies are neutralizing, which is a challenge in SARS-CoV-2 infection in human medicine, as the virus can sometimes evade the immune system. It is noted that antibodies against a specific fraction or protein are sometimes produced but are incapable of neutralization [30].

The primary limitation of this study is its retrospective design, which inherently carries certain biases and constraints. Additionally, the absence of diverse variant strains in the VNT and the receptor-binding domain

enzyme-linked immunosorbent assay (RBD-ELISA) test could impact the comprehensiveness of the findings. Furthermore, the lack of follow-up on seropositive animals limits our understanding of the persistence and implications of SARS-CoV-2 antibodies in European bison populations. However, the RBD used in the ELISA was capable of detecting seropositive samples from different waves of COVID-19 outbreaks in ferrets in Spain, spanning from the first to the seventh wave. On the positive side, the significant volume of samples collected from various regions in Poland and the extensive study duration covering different waves of COVID-19 outbreaks serve as major strengths of this investigation, providing valuable insights into SARS-CoV-2 exposure in European bison populations.

In our study, the limit of detection (LOD) was calculated using animal negative serum samples (collected before the pandemic) as mean + 3 × Standard deviation. LOD was used as the cut-off value to discriminate between positive and negative samples. The proximity to the cut-off could be explained as low exposure or by contrast a decreasing of low antibody levels. However, this hypothesis could not be evaluated due to the absence of a follow-up of the seropositive animals. Related to the sensitivity and specificity of these in-house tests, this test was compared in a human ELISA [29], showing a correlation of 0.8. In this sense, sensitivity and specificity calculated using pre-COVID-19 healthy donors and the convalescent plasma samples were 72% and 100%, respectively. In another study comparing eight different serological tests in cats, this in-house ELISA showed a sensitivity of 90% and a specificity of 98% [31]. Moreover, this in-house ELISA has been included in different epidemiological studies in different animal species (e.g [22, 29]).

Conclusion

In conclusion, this study represents the first report of SARS-CoV-2 seroprevalence in both free-ranging and captive European bison populations in Poland. Based on the findings, the impact of SARS-CoV-2 infection in these ruminants, whether in captive or free-ranging status, appears to be very limited. The most probable route of transmission observed was human-to-European bison transmission, as all seropositive animals were captive animals with documented contact with humans, such as tourists and keepers. These findings highlight the importance of continued vigilance and research to better understand the dynamics of SARS-CoV-2 transmission in both animal and human populations.

Methods

Animals

Between 2017 and 2023, blood samples were collected from 335 European bison (185 females, 150 males)The age of the animals was assessed by experienced veterinarians, based on teeth eruption, and ranged from 0.25 to 25 years old (mean 6.7). Animals derived from Polish captive ($n = 157$) or free-ranging ($n = 178$) herds. Animals were from different parts of Poland– Augustowska Forest ($n = 1$), Bałtów ($n = 3$), Białowieska Forest ($n = 77$), Bieszczady Mountains ($n = 77$), Borecka Forest ($n = 44$), Gdansk Zoological Garden ($n = 1$), Gołuchów ($n = 6$), Kiermusy ($n = 8$), Knyszyńska Forest ($n = 39$), Międzyzdroje ($n = 1$), Niepołomice ($n = 17$), Pszczyna ($n = 49$), Ustroń ($n = 2$), Warsaw Zoological Garden ($n = 5$) (Fig. 1). Samples were collected in 2017 ($n = 12$), 2018 ($n = 91$), 2019 ($n = 42$), 2020 ($n = 35$), 2021 ($n = 65$), 2022 ($n = 36$) and 2023 ($n = 54$).

Serum samples obtained from healthy, non-infected European bison, prior to the pandemic COVID-19 situation (2017–2018), were used as negative controls ($n = 103$). The study group comprised European bison sampled between 2019 and 2023 ($n = 232$) with 109 males and 123 females, and ages ranging from 0.25 to 23 years old, with a mean of 7.56 years.

Samples were collected using the opportunity of planned immobilization (e.g. during testing before translocation or putting collars) or post-mortem examination (animals found dead or culled after obtaining permission from the General Director of Environmental Protection). No animals were specifically immobilized or culled for the purpose of this study.

Blood was collected from the jugular vein using a 1.2 mm diameter needle into 9 ml sterile tubes containing a clot activator (Medlab Products, Raszyn, Poland). The samples were transported immediately to the laboratory at 4°C. Following, centrifugation (3000 g, 10 min), serum was separated and stored at -20°C until further analysis.

Detection of SARS-CoV-2 antibodies by in-house ELISA (RBD-based ELISA)

Antibodies to SARS-CoV-2 were determined by an indirect ELISA for the detection of IgG specific for RBD (Ancestral SARS-CoV-2 strain, Wuhan strain), described previously [32, 33]. Ninety-six–-well plates were coated overnight, at 4 °C with 100 ng RBD protein in phosphate-buffered saline (PBS). Subsequently, the coating solution was removed and the plate was washed three times with 200 µL per well of PBS + TWEEN 0.05% (PBST). 300 µL of PBST containing 3% dry skimmed milk was added to each well as blocking solution. The plate was incubated with blocking solution for 1 h at 37 °C in a moist chamber. 100 µL of European bison sera, diluted 1:100 in PBS

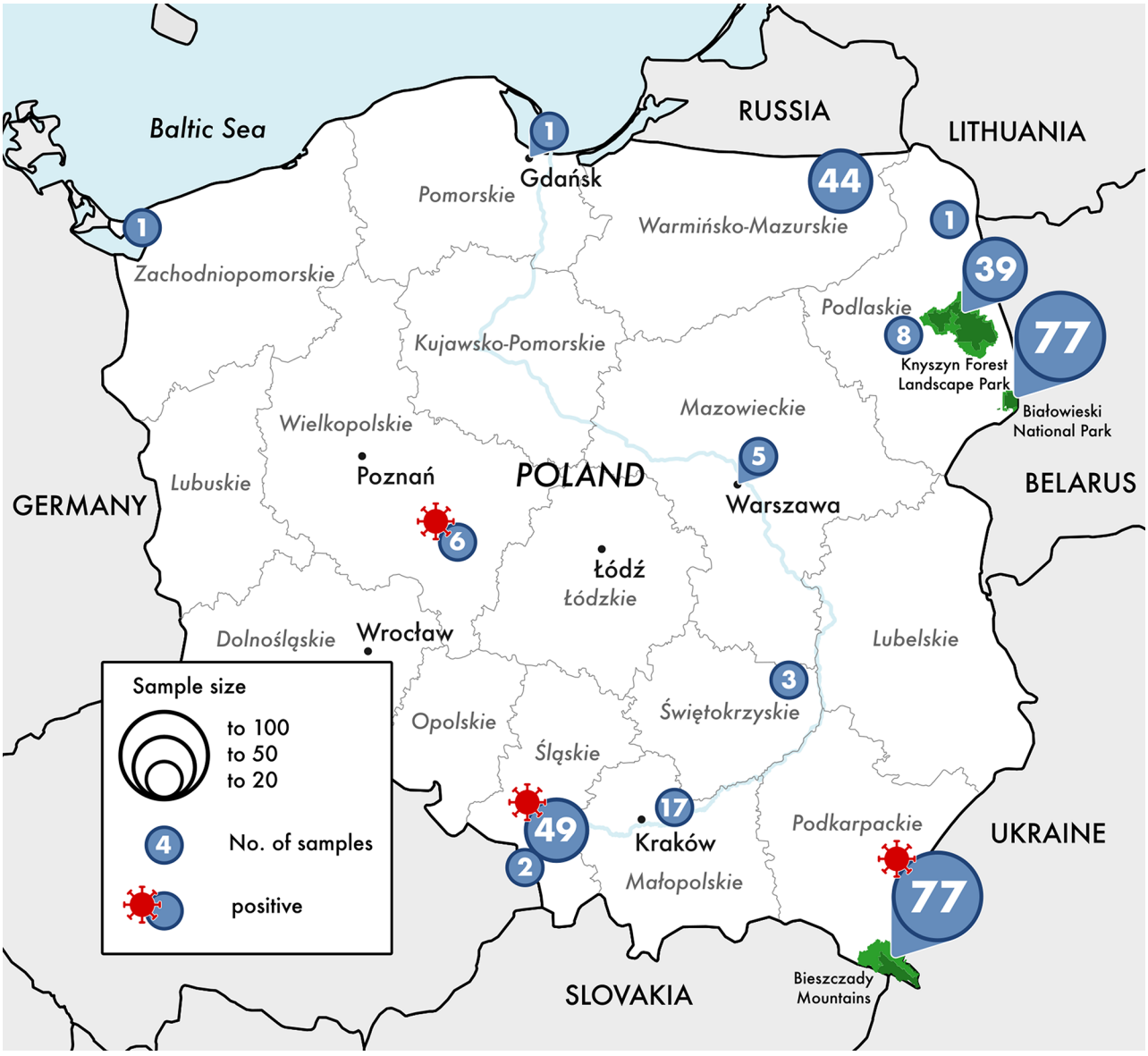

Fig. 1 Map of Poland with number of SARS-CoV-2 serologically tested European bison (*Bison bonasus*)

containing 0.05% Tween 20 and 1% dry skimmed milk (PBSTM), was added to each well. The plates were incubated for 1 h at 37 °C in a moist chamber. After washing the plates for 30 s 6 times with PBST followed by 1 wash with PBS for 1 min, 100 µL/well of Protein A/G conjugated to horseradish peroxidase (Thermo Fisher Scientific, Waltham, MA, USA) diluted 1:100,000 in PBST-M was added per well. The plates were incubated for 1 h at 37 °C in a moist chamber and were washed again with PBST and PBS as described above. The substrate solution (ortho-phenylene-diamine) and stable peroxide substrate buffer (Thermo Fisher Scientific, Waltham, MA, USA) were added at 100 µL per well and developed for 20 ± 5 min at room temperature in the dark. The reaction was stopped by adding 100 µL of 2.5 M H2SO4 to each well. Absorbance values were read at 492 nm. in an automatic micro ELISA reader (ELISA Reader Labsystems Multiskan, Midland, ON, Canada). Each plate included a panel of seropositive samples with a known antibody status from different species such as cat [24, 32], human [30], ferret (*Mustela putorius furo*) [29] and a black-and-white ruffed lemur (*Varecia variegata*) [22], and serum from a healthy, non-infected E. bison obtained before pandemic COVID-19 situation as a negative control. The same positive and negative sera were used for all assays. All samples were run in duplicate. The cut-off was set to 0.290 Optical Density units (OD units) (mean + 3 standard deviations of values from 104 animals obtained before the COVID-19 situation (2017 and 2018) as negative controls) and the results above this value were

considered positive. Control sera were obtained from the collection of sera of the Laboratory of Clinical Immunology at the Faculty of Veterinary Medicine, University of Zaragoza, Spain.

Micro-neutralization assay of SARS-CoV-2 (VNT)

VNT was performed as described previously [24]. The viral strain used for this technique was the B1.1. strain (Alpha variant, B1.1.7) and the BA.5 strain (Omicron variant). The neutralization ID50 was calculated as the highest dilution that protected more than 50% of the wells from the cytopathic effect. This test was performed in serum samples that tested positive in the in-house ELISA for SARS-CoV-2 antibody detection as a complementary technique.

Statistical analysis

Data collected for the entire population were analysed using descriptive statistics. If there were no complete set of data for animal, it was excluded from statistical analysis The data were analyzed using SPSS version 26 software (SPSS Inc., Chicago, USA). A descriptive analysis of the variables was carried out, considering the proportion of qualitative variables (sex, age, and type of population - captive or free-ranging). Fisher's exact test and a 95% confidence interval (CI) were used to compare proportions. In all analyses, the significance level was established at $P < 0.05$. For some European bison, it was not possible to acquire all data; these animals were not considered when performing statistics.

Abbreviations

ACE2	Angiotensin I Converting Enzyme 2
CI	Confidence Interval
COVID-19	Coronavirus Disease 2019
ID	Identification
IUCN	International Union for Conservation of Nature
OD	Optical Density
RBD	Receptor Binding Domain
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
VNT	Virus Neutralization Test

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Author contributions

Conceptualization, AD, MW, WO, KA, and SVS; methodology, AD, VMS, WO, AG, and DM; software, AF, DL and AO; validation, NPF, MA, and MDP; formal analysis, AF, and DM; investigation AD, JED, AG, and MDP; resources, AD and SVS; data curation, AD, VMS, and SVS; writing-original draft preparation, AD, VMS, JED, and DL; writing-review and editing, AD, VMS, JED, and SVS; visualization, AD and SVS; supervision, AD, WO, KA and SVS; funding acquisition, MW, WO, KA, and AO. All authors have read and agreed to the published version of the manuscript.

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Data availability

More detial data is available on request from the authors.

Declarations

Ethics approval and consent to participate

No animal was culled or immobilized for this study. Samples were collected on the occasion when animals were culled or immobilized for other reasons. Any lethal testing was carried out by local institutions responsible for European bison management, and each culling was performed with the necessary permit. The collection and storage of serum samples from dead animals was based on the decision of the Regional Director of Environmental Protection in Warsaw: according to this decision, no permission is needed for the collection of dead animals for scientific purposes. In addition, in accordance with II Local Ethical Committee for Animal Experiments in Warsaw, no approval was needed for ante-mortem sampling, as it was conducted as part of standard veterinary care.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA, et al. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol Nat Res. 2020;5:536–44. https://doi.org/10.1038/s41564-020-0695-z.
2. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG et al. A new coronavirus associated with human respiratory disease in China. Nature 579:265–9. https://doi.org/10.1038/s41586-020-2008-3
3. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Nat Med. 2020;26:450–4. https://doi.org/10.1038/s41591-020-0820-9.
4. Palmer MV, Martins M, Falkenberg S, Buckley A, Caserta LC, Mitchell PK, et al. Susceptibility of white-tailed deer (*Odocoileus virginianus*) to SARS-CoV-2. J Virol. 2021;95(11):e00083–21. https://doi.org/10.1128/JVI.00083-21.
5. Zhang G, Li B, Yoo D, Qin T, Zhang X, Jia Y, et al. Animal coronaviruses and SARS-CoV-2. Transbound Emerg Dis. 2021;68(3):1097–110. https://doi.org/10.1111/tbed.13791.
6. Letko M, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat Microbiol. 2020;5:562–9. https://doi.org/10.1038/s41564-020-0688-y.
7. Damas J, Hughes GM, Keough KC, Painter CA, Persky NS, Corbo M, et al. Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates. Proc Natl Acad Sci U S A. 2020;117:22311–22. https://doi.org/10.1073/pnas.2010146117.
8. Plumb G, Kowalczyk R, Hernandez-Blanco JA. *Bison bonasus*. The IUCN Red List of Threatened Species 2020. 2020;e:T2814A45156279. https://doi.org/10.2305/IUCN.UK.2020-3.RLTS.T2814A45156279.en
9. Krajewska-Wędzina M, Krzysiak MK, Bruczyńska M, Orłowska B, Didkowska A, Radulski Ł, et al. Ten years of animal tuberculosis monitoring in Free-living European Bison (*Bison bonasus*) in Poland. Anim (Basel). 2023;13(7):1205. https://doi.org/10.3390/ani13071205.
10. Kwiecień E, Stefańska I, Kizerwetter-Świda M, Chrobak-Chmiel D, Didkowska A, Bielecki W, et al. Prevalence and Genetic Diversity of *Trueperella pyogenes* isolated from infections in European Bison (*Bison bonasus*). Anim (Basel). 2022;12(14):1825. https://doi.org/10.3390/ani12141825.
11. Didkowska A, Orłowska B, Krajewska-Wędzina M, Krzysiak M, Bruczyńska M, Wiśniewski J, et al. Intra-palpebral tuberculin skin test and Interferon Gamma Release Assay in Diagnosing Tuberculosis due to *Mycobacterium caprae* in

European Bison (*Bison bonasus*). Pathogens. 2022;11:260. https://doi.org/10.3390/pathogens11020260.
12. Klich D, Didkowska A, Pyziel-Serafin AM, Perlińska-Teresiak M, Wołoszyn-Gałęza A, Żoch K, et al. Contact between European bison and cattle from the cattle breeders' perspective, in the light of the risk of pathogen transmission. PLoS ONE. 2023;18(5):e0285245. https://doi.org/10.1371/journal.pone.0285245.
13. Olech W, Klich D, Perzanowski K. Development of a new Action Plan for the European bison. Oryx. 2019;53(2):214–4. https://doi.org/10.1017/S0030605318001369.
14. Pomorska-Mól M, Turlewicz-Podbielska H, Gogulski M, Ruszkowski JJ, Kubiak M, Kuriga A, et al. A cross-sectional retrospective study of SARS-CoV-2 seroprevalence in domestic cats, dogs and rabbits in Poland. BMC Vet Res. 2021;17(1):322. https://doi.org/10.1186/s12917-021-03033-2.
15. Kaczorek-Łukowska E, Wernike K, Beer M, Wróbel M, Małaczewska J, Mikulska-Skupień E, et al. High seroprevalence against SARS-CoV-2 among dogs and cats, Poland, 2021/2022. Anim (Basel). 2022;12(16):2016. https://doi.org/10.3390/ani12162016.
16. Suligowski R, Ciupa T. Five waves of the COVID-19 pandemic and green-blue spaces in urban and rural areas in Poland. Environ Res. 2023;216(Pt 3):114662. https://doi.org/10.1016/j.envres.2022.114662.
17. Tewari D, Miller R, Livengood J, Wang L, Killian ML, Bustamante F, et al. SARS-CoV-2 Infection Dynamics in the Pittsburgh Zoo Wild felids with two viral variants (Delta and Alpha) during the 2021–2022 pandemic in the United States. Anim (Basel). 2023;13(19):3094. https://doi.org/10.3390/ani13193094).
18. Oreshkova N, Molenaar RJ, Vreman S, Harders F, Oude Munnink BB, Hakze-van der Honing RW et al. I. SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. Euro Surveill 2021;25(23):2001005. doi: 10.2807/1560-7917.ES.2020.25.23.2001005. Erratum in: Euro Surveill.
19. McAloose D, Laverack M, Wang L, Killian ML, Caserta LC, Yuan F, et al. From people to Panthera: natural SARS-CoV-2 infection in Tigers and Lions at the Bronx Zoo. mBio. 2020;11(5):e02220–20. https://doi.org/10.1128/mBio.02220-20.
20. Nagy A, Stará M, Vodička R, Černíková L, Jiřincová H, Křivda V, et al. Reverse-zoonotic transmission of SARS-CoV-2 lineage alpha (B.1.1.7) to great apes and exotic felids in a zoo in the Czech Republic. Arch Virol. 2022;167(8):1681–5. https://doi.org/10.1007/s00705-022-05469-9.
21. Dusseldorp F, Bruins-van-Sonsbeek LGR, Buskermolen M, Niphuis H, Dirven M, Whelan J, et al. SARS-CoV-2 in lions, gorillas and zookeepers in the Rotterdam Zoo, the Netherlands, a One Health investigation, November 2021. Euro Surveill. 2023;28(28):2200741. https://doi.org/10.2807/1560-7917.ES.2023.28.28.2200741.
22. Musoles-Cuenca B, Aguiló-Gisbert J, Lorenzo-Bermejo T, Canales R, Ballester B, Romani-Cremaschi U, et al. Molecular and serological studies on potential SARS-CoV-2 infection among 43 lemurs under Human Care-evidence for past infection in at least one individual. Anim (Basel). 2023;31(1):140. https://doi.org/10.3390/ani14010140.
23. Decaro N, Vaccari G, Lorusso A, Lorusso E, De Sabato L, Patterson E, et al. Possible human-to-dog transmission of SARS-CoV-2, Italy, 2020. Emerg Infect Dis. 2021;27(7):1981–4. https://doi.org/10.3201/eid2707.204959.
24. Villanueva-Saz S, Martínez M, Rueda P, Bolea S, Pérez MD, Verde M, et al. The dynamics of neutralizing antibodies against SARS-CoV-2 in cats naturally exposed to virus reveals an increase in antibody activity after re-infection. Vet Res Commun. 2023;47(4):2179–84. https://doi.org/10.1007/s11259-023-10087-0.
25. Hamer SA, Nunez C, Roundy CM, Tang W, Thomas L, Richison J, et al. Persistence of SARS-CoV-2 neutralizing antibodies longer than 13 months in naturally infected, captive white-tailed deer (*Odocoileus virginianus*), Texas. Emerg Microbes Infect. 2022;11(1):2112–5.
26. Villanueva-Saz S, Giner J, Fernández A, Lacasta D, Ortín, Ramos JJ, et al. Absence of SARS-CoV-2 antibodies in natural environment exposure in Sheep in Close Contact with humans. Anim (Basel). 2021;11(7). https://doi.org/10.3390/ani11071984.
27. Palmer MV, Martins M, Falkenberg S, Buckley A, Caserta LC, Mitchell PK, et al. Susceptibility of white-tailed deer (*Odocoileus virginianus*) to SARS-CoV-2. J Virol. 2021;201(11):e00083–21. https://doi.org/10.1128/JVI.00083-21.
28. Wernike K, Aebischer A, Michelitsch A, Hoffmann D, Freuling C, Balkema-Buschmann A, et al. Multi-species ELISA for the detection of antibodies against SARS-CoV-2 in animals. Transbound Emerg Dis. 2021;68(4):1779–85. https://doi.org/10.1111/tbed.13926.
29. Giner J, Lebrero ME, Trotta M, Rueda P, Vilalta L, Verde M et al. (2024) Seroprevalence of anti-SARS-CoV-2 antibodies in household domestic ferrets (*Mustela putorius furo*) in Spain, 2019–2023. Vet Res Commun 2024;48(1):533–540. https://doi.org/10.1007/s11259-023-10190-2
30. Santiago L, Uranga-Murillo I, Arias M, González-Ramírez AM, Macías-León J, Moreo E, et al. Determination of the concentration of IgG against the Spike receptor-binding domain that predicts the viral neutralizing activity of Convalescent plasma and serum against SARS-CoV-2. Biology (Basel). 2021;10(3):208. https://doi.org/10.3390/biology10030208.
31. Diezma-Díaz C, Álvarez-García G, Regidor-Cerrillo J, Miró G, Villanueva-Saz S, Dolores Pérez M et al. (2023). A comparative study of eight serological methods shows that spike protein-based ELISAs are the most accurate tests for serodiagnosing SARS-CoV-2 infections in cats and dogs. Front Vet Sci 2023;10:1121935. https://doi.org/10.3389/fvets.2023.1121935)
32. Giner J, Villanueva-Saz S, Tobajas AP, Pérez MD, González A, Verde M, et al. SARS-CoV-2 Seroprevalence in Household Domestic ferrets (*Mustela putorius furo*). Anim (Basel). 2021;11(3):667. https://doi.org/10.3390/ani11030667.
33. Villanueva-Saz S, Giner J, Tobajas AP, Pérez MD, González-Ramírez AM, Macías-León J, et al. Serological evidence of SARS-CoV-2 and co-infections in stray cats in Spain. Transbound Emerg Dis. 2021;69(3):1056–64. https://doi.org/10.1111/tbed.14062.

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