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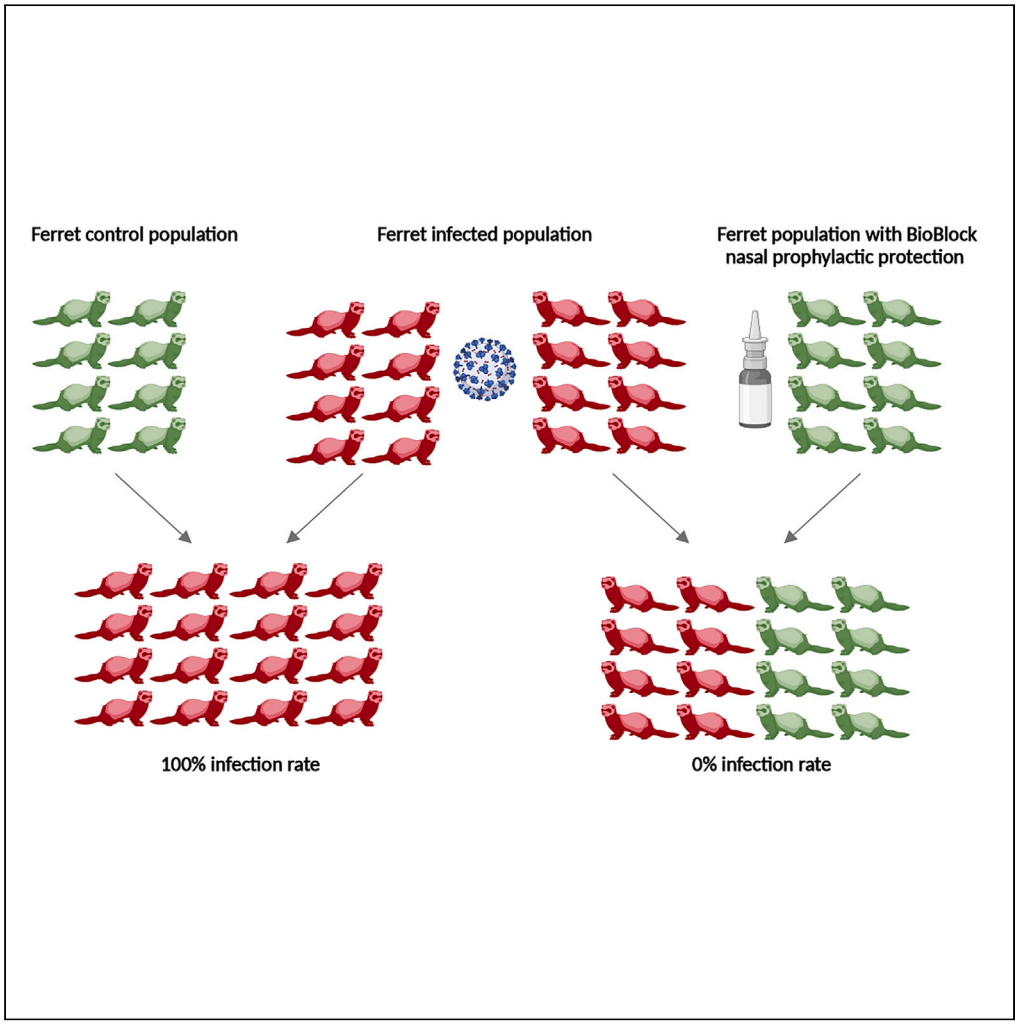


Kiira Gildemann, Mari-Liis Tsernant, Laura Liivand, ..., Mart Ustav, Jr., Mart Ustav, Joachim Matthias Gerhold

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## Highlights

Bovine colostrum-derived antibodies can neutralize SARS-CoV-2

These neutralizing antibodies work on different variants of concern

The antibodies were formulated into a nasal spray

The spray solution administered to ferrets blocked virus transmission 100%

Gildemann et al., iScience 27, 110326
July 19, 2024 
https://doi.org/10.1016/j.isci.2024.110326

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# Anti-SARS-CoV-2 antibodies in a nasal spray efficiently block viral transmission between ferrets

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## SUMMARY

**Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to spread in the population. We recently reported the production of bovine colostrum-derived antibodies that can neutralize the virus. These have been formulated into a nasal spray. The immunoglobulin preparation is capable of blocking interaction of the trimeric spike protein (Tri S) of SARS-CoV-2 with the cellular receptor angiotensin-converting enzyme 2 (ACE2), entry of a pseudovirus carrying the Tri S into ACE2 over-expressing human embryonic kidney (HEK) cells, and entry of the virus into live Vero E6 cells. Using an ELISA assay, we demonstrate here that this holds true for different SARS-CoV-2 variants of concern. Using the ferret transmission model, we show that the nasal spray formulation of anti-SARS-CoV-2 immunoglobulins efficiently blocks transmission of SARS-CoV-2 from infected to uninfected ferrets. The results indicate that the use of the nasal spray in humans can add an effective additional layer of protection against the virus, and might be applicable for other viruses of the upper respiratory tract.**

## INTRODUCTION

Vaccination, naturally acquired immunity, and social distancing have contributed to ending the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. Yet endemic transmission of the virus continues, making the quest for additional countermeasures, such as antiviral therapeutics, an urgent priority.[1] SARS-CoV-2 presents a trimeric spike protein (Tri S) on its surface. Binding of this protein, specifically of the receptor binding domain (RBD) of the spike protein 1 (S1) subunit, to the cellular receptor protein angiotensin-converting enzyme 2 (ACE2) triggers the viral entry cascade, ultimately resulting in infection.[2] Neutralizing antibodies (nAb) have the potential to halt this process by binding to the SARS-CoV-2 Tri S protein, sterically preventing its interaction with ACE2 and, consequently, blocking entry of SARS-CoV-2 into cells.[3]

We recently reported the use of bovine colostrum-derived nAb as a prophylactic agent against SARS-CoV-2[4] and a resulting nasal spray formulation called BioBlock, which has been in use in the Estonian population since spring 2021. The preparation and quality control of the colostrum-derived antibody formulation was described in detail in our previous report.[4] In brief, defatted and casein-depleted colostrum was incubated at a low pH of 3.3 to inactivate potential viral contaminants. The immunoglobulin (Ig)-enriched fraction was then prepared by precipitation with 2 M ammonium sulfate, and the precipitated proteins (mainly Igs) were dissolved in 1× DPBS (Dulbecco's Phosphate Buffered Saline). The preparation was further dialyzed against 1× DPBS, sterile filtered, pasteurized, and evaluated for protein integrity. Concentration was determined using SDS-PAGE and measurement of UV absorbance at 280 nm. After final formulation, the product was evaluated for pH, viscosity, polydispersity index, and sterility.[4] The nAbs contained in the nasal spray formulation were derived from the colostrum of cows immunized with the spike proteins of SARS-CoV-2. *In vitro*, they showed very high efficacy in blocking the interaction of Tri S and ACE2 and inhibited viral entry in three different experimental setups. We hypothesized that the presence of the designed formulation in the nasal mucosa would prevent virus docking to ACE2 of mucosal cells and thereby block viral entry through the nasal cavity. If correct, prophylactic administration of the nAb should also inhibit transmission of SARS-CoV-2. In a mouse model, intra-nasal administration of a human-derived monoclonal antibody indeed inhibited infection with SARS-CoV-2.[5]

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https://doi.org/10.1016/j.isci.2024.110326

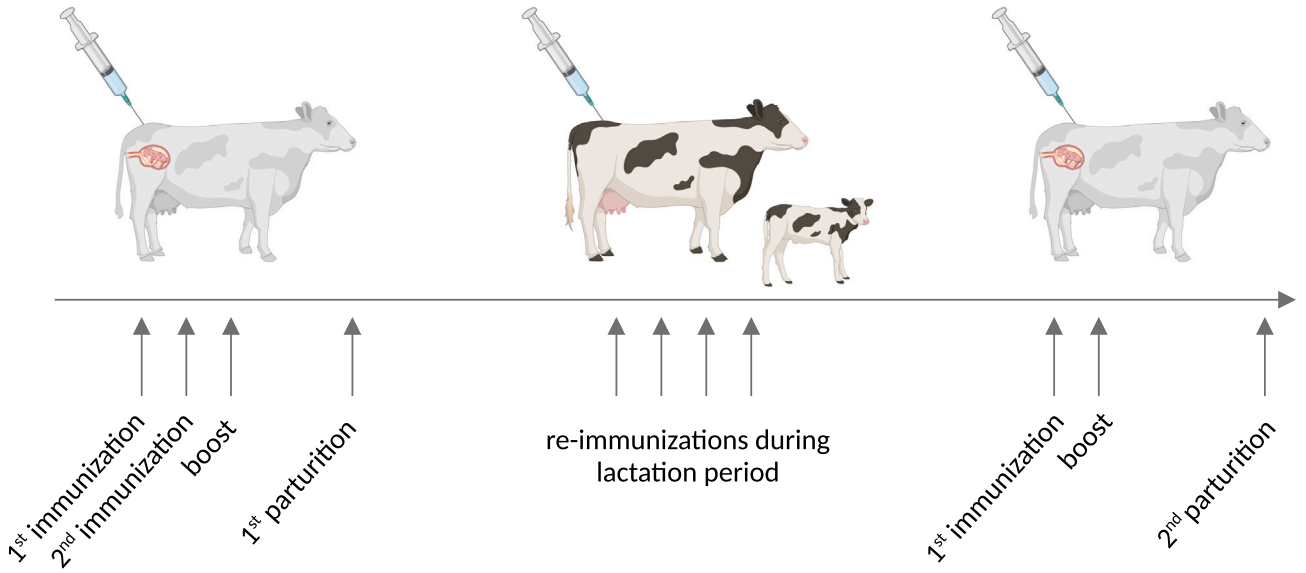


**Figure 1. Immunization scheme including re-immunizations of cows**

Animals were initially immunized during the third trimester of pregnancy about 60, 21, and 14 days before parturition. Cows were then further immunized about every 5–8 weeks during the lactation/gestation period. In the third trimester of the next pregnancy, about 42 and 14 days before parturition, the next immunizations were conducted.

Ferrets have evolved as a leading animal model for the evaluation of vaccines and therapeutics against respiratory virus infections including influenza viruses,[6] pneumo- and paramyxoviruses,[7] and SARS-CoV-2.[8,9] Contributing to high predictive power of the model are similar relative proportions of the ferret and human upper and lower respiratory tract and comparable density of submucosal glands in the bronchial wall and generations of terminal bronchioles.[7] Infection of ferrets with SARS-CoV-2 results predominantly in upper respiratory disease with high virus shedding and efficient transmission, resembling disease presentation in the majority of human adults.[10]

In this study, we investigated whether BioBlock is capable of blocking the transmission of SARS-CoV-2 from infected to uninfected ferrets.

We demonstrate that the previously characterized nasal spray formulation of BioBlock, containing colostrum-derived anti-SARS-CoV-2 neutralizing antibodies, is highly effective in suppressing transmission of the virus in a population containing both infected and uninfected subjects. We show that such polyclonal preparations are highly efficacious against newly emerged virus variants. The re-vaccination scheme used for cows results in neutralizing antibodies against SARS-CoV-2 that are effective against highly mutated novel SARS-CoV-2 strains, which were not previously known and therefore not included as antigens during the original immunizations.

## RESULTS

Cows in the third trimester of pregnancy were immunized with a total of three injections of either the SARS-CoV-2 RBD or Tri S. The initial immunization took place at approximately 60 days before the calculated date of parturition, a first booster was administered 21 days later, and a final booster was given approximately 2 weeks before birth of the calf.[4] We thereafter continued immunizations with a mixture of SARS-CoV-2 variants of concern (VOC) Alpha, Beta, and Gamma-derived RBD proteins throughout the lactation period (commonly 9–10 months), at intervals of 5–8 weeks, delivering a total amount of 0.1 mg of protein mixture (Figure 1). The cows were re-impregnated 2 months after birth of the calf. When VOC Delta emerged and became predominant, the immunization strategy was changed to using 0.1 mg of VOC Delta-derived S1 protein for re-immunizations. Six weeks before the 2nd parturition, immunization was conducted with 0.1 mg of the VOC Delta RBD and about 3 weeks prior to expected calving with 0.3 mg of VOC Delta S1 protein (Figure 1).

### A colostrum-derived Ig preparation from re-immunized cows is highly efficient in blocking ACE2 and SARS-CoV-2 trimeric spike interaction

We prepared Igs from colostrum according to our previously described procedure[4] and validated efficacy *in vitro*, using an inverted enzyme-linked immunosorbent assay (ELISA) protocol. We found that neutralization capacity coincided well with pseudovirus and cytopathic effect-based neutralization assays.[4] The ELISA test makes use of the interaction of the Tri S with ACE2. In the absence of neutralizing antibodies, signals (expressed as optical density [OD] at a wavelength of 450 nm; OD450) were strong, since labeled ACE2 bound to the Tri S protein. As neutralizing antibodies emerged in the sample preparation, signals gradually decreased, resulting in very low intensities (OD450) as high concentrations of neutralizing antibodies were present. We coated ELISA plates with Tri S proteins of Alpha, Delta, or Omicron strains and subjected the Ig preparation of the re-immunized cows and a non-immunized cow to analyses (Figure 2). We observed that half-maximal

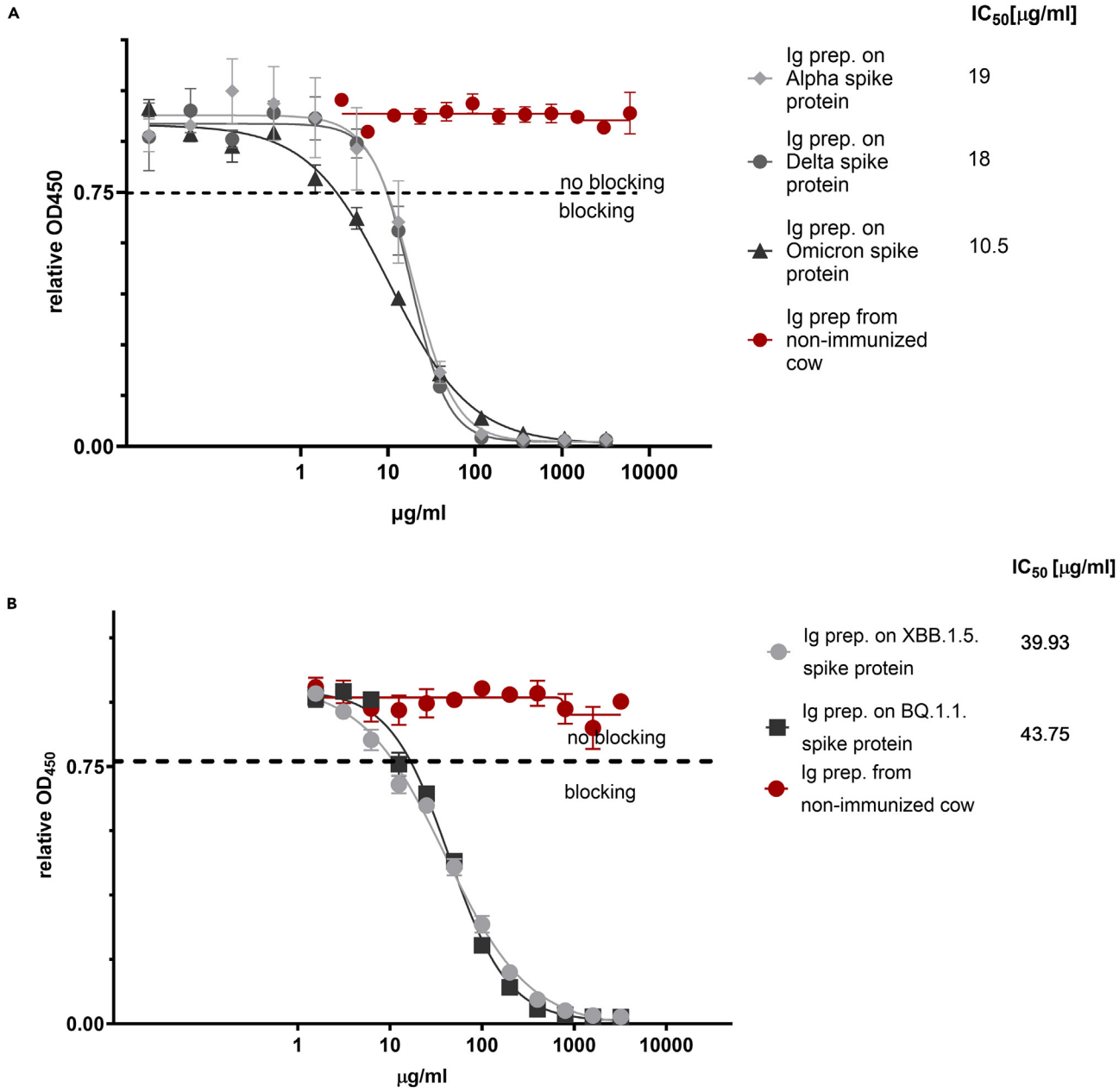


**Figure 2. The Ig preparation retrieved after several reimmunizations shows high potency in blocking the *in vitro* interaction of ACE2 with the Tri S proteins of Alpha, Delta, Omicron, and VOC strains of SARS-CoV-2**

(A) Activity of the Ig preparation against the Tri S proteins of the Alpha, Delta, and Omicron strains of SARS-CoV-2 *in vitro*. In contrast, the preparation derived from colostrum of a non-immunized cow did not show any inhibition of the interaction between ACE2 and Tri S *in vitro*.

(B) Activity of the Ig preparation against VOC BQ.1.1 and XBB.1.5. Employing our inverted ELISA analyses, we observed good efficacy of the nAbs in blocking Tri S–ACE2 interaction. Half-maximal inhibition ($IC_{50}$) was determined through a non-linear regression analysis with a variable slope (four parameters). XY-plots for the Ig preparation analyses are presented with non-linear fit and standard deviations (SD); $n = 3$ replicates.

inhibition ($IC_{50}$) was 10–20 µg/mL depending on the analyzed VOC. This was in a much lower range than we had observed with the initial Ig preparation from previous immunizations employing the same assay,[4] indicating maturation of immune responses due to repetitive boosting doses (Figure 2A). Most importantly, the quality of the immune response improved. All immunizations were done with antigens from VOC Alpha, Beta, Gamma, and Delta, but the subsequently emerged VOC Omicron was also potently neutralized. VOC Omicron has more than 50 mutations compared to the original SARS-CoV-2 strain, 32 of which are located in the spike protein and 26 are unique to Omicron compared to the VOCs that served as antigens.[11]

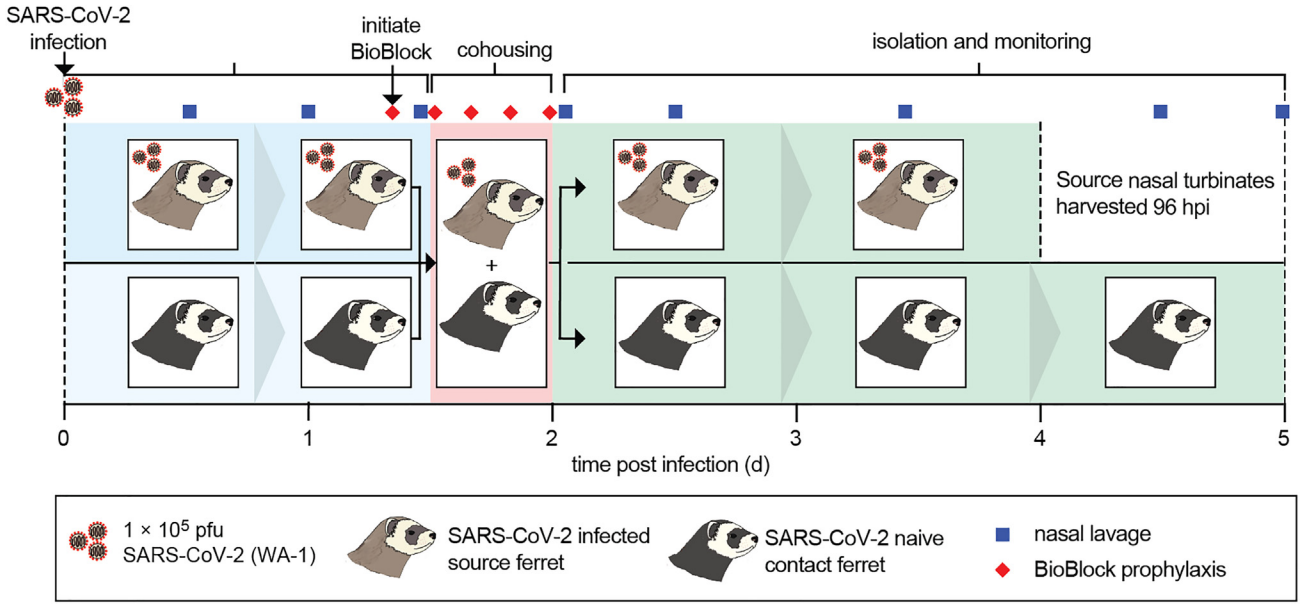


**Figure 3. Experimental setup for ferret experiments**

Depicted are uninfected (dark gray; contact) and infected (light gray; source) ferrets. Blue background indicates the time uninfected and infected ferrets were housed separately. Source ferrets for SARS-CoV-2 were infected 1.5 days prior to cohousing. Nasal lavages of infected source animals and naive contact ferrets were taken every 12 h (blue squares). Four hours prior to cohousing of source and contact animals, a first dose of BioBlock and buffer alone (red rhombs), respectively, were administered. During cohousing (pink background) contact ferrets were treated with BioBlock or buffer alone every 4 h (red rhombs). After 12 h animals were separated and monitored further (green background). After termination of the experiment, nasal turbinates were harvested. The duration of the experiment in days is indicated below the colored boxes.

We further tested the Ig preparation from the colostrum of re-immunized cows for *in vitro* efficacy against the Tri S proteins of the VOC Omicron 2 isolates BQ.1.1 and XBB.1.5 (Figure 2B), which evolved after the initial appearance of Omicron and carried even more differences in the spike protein than the initial Omicron isolate.[12,13] Albeit at somewhat lower efficiency compared to neutralization of VOC Alpha, Delta, and Omicron 1, the preparation was still very potent in inhibiting VOC Omicron 2-derived Tri S–ACE2 interactions, showing $IC_{50}$ values of 44 and 40 μg/mL for the BQ.1.1 and XBB.1.5 isolates, respectively.

## A nasal spray containing colostrum-derived nAbs against SARS-CoV-2 blocks transmission of the virus with high efficacy

Since we had previously formulated the Ig preparation of immunized cows into a nasal spray (BioBlock) and had shown the component antibodies to persist on the human nasal mucosa for at least 4 h,[4] we next employed the ferret model (Figure 3) to test whether a prophylactic nasal spray administration of BioBlock would prevent SARS-CoV-2 transmission to healthy subjects, recapitulating a community transmission scenario in which people are in close contact with infected individuals for a defined period of time. We conducted the study in two replicates and treated a total of 8 ferrets with the nasal spray formulation at 0.236 mg/kg. Eight control animals received the nasal spray vehicle without the Ig preparation. After an initial nasal lavage with either the colostrum-derived nasal spray formulation or vehicle alone 4 h prior to co-housing, each treated animal was housed with a SARS-CoV-2 (WA-1 strain)-infected source animal (source to sentinel ratio 1:1) in a direct-contact setting. Treatment of the contacts with the spray-formulation BioBlock was continued in 4-h intervals for the duration of co-housing (12 h total). Subsequently, both donor and recipient animals were monitored for another 96 h, followed by extraction of respiratory tissues and determination of infectious viral titers and viral RNA copies. The study timeline in shown in Figure 3.

We observed that nasal lavages of source animals (vehicle source and treated source) showed high titers of SARS-CoV-2 (50% tissue culture infectious dose [$TCID_{50}$] of $10^5$ two days post infection) (Figure 4A). The buffer-treated sentinels started to show measurable titers ($TCID_{50}$ of $10^{2.5}$) 2 days post infection (dpi) and high titers ($TCID_{50}$ of $10^5$ to $10^6$) 6 dpi. These findings were corroborated by viral RNA copies present in nasal lavages (Figure 4B), which reached $10^5$–$10^6$ copies 1–4 dpi. Vehicle-treated contacts turned PCR-positive ($10^2$ RNA copies) 1 dpi and developed high viral load ($10^6$ copies) 6 dpi (Figure 4B).

Contrary to efficient viral spread to vehicle-treated contacts, all sentinels that had received BioBlock were not productively infected—neither developing measurable viral titers nor showing a rise in viral RNA copies (Figures 4A and 4B)—confirming efficient protection by BioBlock against viral transmission from infected to uninfected subjects *in vivo*.

At study end, viral titers and viral RNA copy numbers were determined in nasal turbinates extracted from all animals (Figures 4C and 4D). We observed high virus burden in all source- and vehicle-treated contact animals, whereas turbinates of sentinels that had received BioBlock

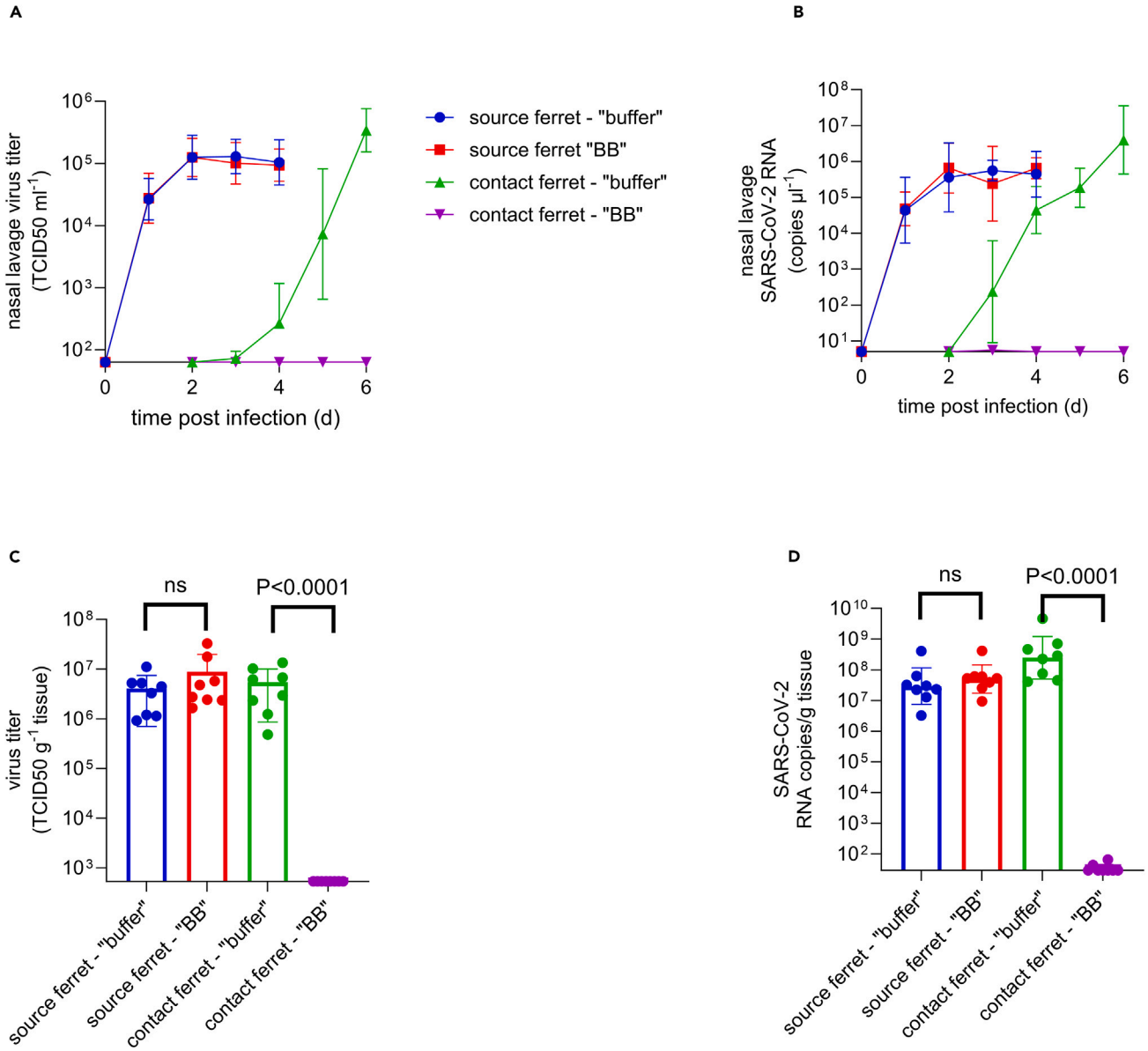


**Figure 4. The Ig preparation effectively inhibits transmission of SARS-CoV-2 from infected to uninfected ferrets**

(A) Virus titers in nasal lavages from animals carrying SARS-CoV-2 (source ferret) and those exposed to the virus (contact ferret) are depicted. One day after infection, source animals showed high titers of the virus with a further elevation on day 2. Titers stayed high until the animals were terminated. BioBlock-treated animals were monitored from day 2 until day 6 (termination of the experiment). Green triangles and line depict the buffer-alone treated animals and show a clear rise in viral titer on day 4 which rapidly climbs until day 6. During the same period, animals treated with BioBlock-containing solution (purple triangles and line) did not reveal a significant rise in viral titer.

(B) RNA copy numbers of the nasal lavages confirmed viral titration results. After termination of the experiment, nasal turbinates were collected.

(C and D) Source ferrets (blue bars) and buffer-treated contact animals (green bars) showed high SARS-CoV-2 titers (C) and RNA copy numbers (D). Source animals (red bars) of ferrets to be co-housed with BioBlock-treated animals were likewise efficiently infected, but prophylactic BioBlock suppressed transmission to contacts (magenta bars). XY-plots in Figures 4A and 4B are presented with geometric SD. Data presented in Figures 4C and 4D were not normally distributed and thus log transformed. Ordinary one-way ANOVA was then used with Brown-Forsythe test for equal variances. Correction for multiple comparisons was conducted using Šidàk's test. For statistical analyses $p$ values $< 0.05$ were considered to be statistically significant. Data in boxplots are presented as mean with SD. $n = 8$ animals for each treatment (total 32).

prophylaxis were virus-free. This finding fully supported the conclusion that intranasal treatment of ferrets with prophylactic BioBlock fully protected against SARS-CoV-2 transmission.

## DISCUSSION

We demonstrate in this study that anti-SARS-CoV-2 nAb derived from bovine colostrum are highly effective against a range of SARS-CoV-2 VOC and potently block viral spread from infected source animals to prophylactically treated contact animals in the ferret transmission model. Previously, we established the high potential of the bovine Ig preparation *in vitro*.[4] However, this achievement was restricted to the original Wuhan strain and closely related VOC Alpha, Beta, Gamma, and Delta. This first generation of VOC was replaced in less than a year by the newly emerged VOC Omicron lineage, which is considerably different from the original SARS-CoV-2 isolate and VOC Alpha, Beta, Gamma, and Delta.[11]

Although the efficacy of the initial Ig preparation against different VOC was encouraging, we still chose to re-immunize cows that had given birth to calves after initial immunization. We did so throughout the entire lactation period. As new variants emerged sequentially, we reacted and conducted immunizations with the respective VOC as they emerged (Alpha, Beta, Gamma, and Delta). The cows had been re-impregnated, which enabled us to re-apply the initial immunization scheme prior to birth of the calf, in which we immunized and boosted during the third trimester of pregnancy. This immunization was done using the Delta spike protein. Coincidently, this was the time that Omicron emerged. Here we showed that the formulation prepared without known Omicron antigens was also very effective against different Omicron lineages, whose spike protein has numerous mutations compared to the antigens used to immunize cows.[11] This is quite different from what has been seen with anti-SARS-CoV-2 therapeutic monoclonal antibodies, as the antibodies created against pre-Omicron variants mostly lost their potency when Omicron and its sub-lineages emerged.[12,13] Although not demonstrated experimentally yet, this observation further supports the theory that the polyclonal nature of the Ig preparation may be the main contributing factor in the broad neutralizing capacity in comparison to monoclonal antibodies. It seems less likely that the existence of one "super-broad" neutralizing antibody species in the colostrum ensures its extraordinary capability. Perhaps more importantly, cows immunized with antigens from "old" virus strains also developed a protective response against "new" virus strains that were not yet known when this immunization took place. More generally, this would provide an exceptional opportunity to develop effective protective formulations against mutated pathogens even before changes occur and spread in the infected population. Such an approach, if implemented widely and in a timely manner, could offer a significant epidemiological benefit by controlling (restricting) community transmission. Thus, our results provide proof-of-concept that colostrum-derived polyclonal antibody preparations may have superior practical value in situations that call for efficient control of a rapidly changing respiratory pathogen.

Through measures like social distancing, mask-wearing, and hand hygiene, the spread of a virus can be reduced.[14] This prevents new infections and lowers the overall burden of the disease in the community. Controlling community transmission helps to break the chain of infection, lowering the reproduction number (R0) of the virus.[15] This, in turn, leads to a decrease in the number of cases and the potential for outbreaks. Furthermore, controlled transmission helps to avoid overwhelming healthcare systems.[16] Lower case numbers mean that hospitals and healthcare facilities can better manage and provide adequate care for those infected without being stretched beyond capacity. Controlling transmission is also crucial for protecting vulnerable populations such as the elderly, immunocompromised individuals, and those with underlying health conditions.[17] These groups are more susceptible to severe outcomes from viral infections. Some viral infections can lead to long-term health complications even in individuals with mild or asymptomatic cases.[18] Controlling transmission reduces the overall burden of disease and helps minimize these secondary health effects. In addition, controlling community transmission supports vaccination efforts.[19] Vaccines are more effective in preventing severe outcomes and reducing transmission when implemented in a population with lower baseline infection rates. As such, the prophylactic function of BioBlock may significantly impact the epidemiology of SARS-CoV-2 as use of the spray is intended to prevent spreading of the virus.

### Limitations of the study

In order to assess the occurrence of nAbs, a surrogate ELISA was employed. Although evaluated and previously successfully used in our studies, care should be taken in interpreting the results. The ELISA used in this study was corroborated in our earlier study by a pseudovirus assay and a cytopathic cell-based assay. Both showed similar results for the immunoglobulin preparation as did the ELISA method. However, the accepted gold standard in the field is the plaque reduction neutralization test (PRNT).[20] Still, several limitations are discussed for the PRNT, among them scalability (low throughput), long turnaround times, and the requirement of BSL3 (biosafety level 3) facilities and qualified personnel.[20] Since we showed consistent results for the ELISA method employed in this study,[4] we are confident that our surrogate assay delivers credible results. As discussed elsewhere, heparin in blood sample preparations can affect the results of SARS-CoV-2 nAbs detection.[20] Since neither the Ig preparations nor BioBlock contain heparin or have been in contact with it, it is not expected that the results produced by our surrogate assays are compromised in any way.

We acknowledge that the number of test animals (a total of 32) might be considered small. In fact, for each transmission study, we employed a total of 8 treated ferrets (4 treated animals, 4 animals administered with the spray buffer only, and 2 replicates). Ferrets have been established as a large animal model of SARS-CoV-2 upper respiratory tract infection and transmission. They are a non-rodent USDA (United States Department of Agriculture)-controlled species. Group sizes used in this study are consistent with previously published work using this model (see, for e.g., study by Cox et al.[21]). Raw data in this study were subjected to full statistical analysis and were found to be statistically significant. Yet, the design of studies involving large research animals is complex and requires careful evaluation of the potential additional knowledge-gain versus animal welfare concerns and bioethics considerations. Our selected study design represents a scientifically justified compromise between these divergent interests that was approved by all stakeholders including the research scientists and the responsible IACUC (Institutional Animal Care and Use Committee).

The results presented here show that the nasal spray treatment protected uninfected animals under conditions that mimic the situation in the real population of interest. The method of infection was not artificial administration of large amounts of viral particles, but natural transmission from infected animals. However, we also clearly understand the limitations of the model used, and that the evidence presented here derives from *in vitro* assays and animal experiments. The only way to confirm the actual protective effect in humans is through a clinical trial with a control group in conditions where the viral infection rate is high enough to reliably demonstrate the effect of the preparation. Nonetheless, the clinical potential of the approach is supported by the ferret model, which revealed complete protection against infection by intranasally delivered prophylactic BioBlock.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
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  - SARS-CoV-2 neutralizing antibody ELISA
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## ACKNOWLEDGMENTS

We thank the Georgia State University Department of Animal Resources and High Containment Core for Assistance. This study was supported by funding from Enterprise Estonia, grant number 2014-2020.4.02.21-0317.

## AUTHOR CONTRIBUTIONS

Conceptualization: J.M.G., M.U., M.U.J., A.M., K.G., and R.K.P. Methodology: R.K.P., M.U.J., J.M.G., and A.M. Formal analysis: R.K.P., R.M.C., A.M., J.M.G., and K.G. Investigation: R.K.P., R.M.C., J.D.W., C.M.L., and R.E. Resources: K.G., M.L.-T., L. Liivand, V.P., L. Lepasalu, S.R., and A.K. Writing – original draft: J.M.G., K.G., and A.M. writing – review and editing: R.K.P., M.U., M.U.J., A.K., J.M.G., K.G., and A.M. Visualization: R.M.C., A.M., J.M.G., and K.G. Supervision: J.M.G., A.M., M.U.J., and R.K.P. Project administration: J.M.G. Funding acquisition: M.U. and M.U.J.

## DECLARATION OF INTERESTS

The drug product used in this study is marketed and sold in Estonia since 2021.

This study was supported by the funding from Enterprise Estonia grant number 2014-2020.4.02.21-0317.

The use of bovine colostrum as a prophylactic agent against SARS-CoV-2 has been patented (US patent application no 63/160,833).

Work at Georgia State University for this study was carried out under a contract agreement from Icosagen. R.K.P. reports research contract testing from Enanta Pharmaceuticals and Atea Pharmaceuticals, and research support from Gilead Sciences, outside of the described work. R.M.C. reports consulting for Merck & Co, outside of the described work.

Received: January 19, 2024
Revised: April 17, 2024
Accepted: June 18, 2024
Published: June 20, 2024

## REFERENCES

1. Li, G., Hilgenfeld, R., Whitley, R., and De Clercq, E. (2023). Therapeutic strategies for COVID-19: progress and lessons learned. Nat. Rev. Drug Discov. *22*, 449–475. https://doi.org/10.1038/s41573-023-00672-y.
2. Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., Donovan, M., Woolf, B., Robison, K., Jeyaseelan, R., et al. (2000). A Novel Angiotensin-Converting Enzyme–Related Carboxypeptidase (ACE2) Converts Angiotensin I to Angiotensin 1–9. Circ. Res. *87*, e1–e9. https://doi.org/10.1161/01.RES.87.5.e1.
3. Chen, Y., Zhao, X., Zhou, H., Zhu, H., Jiang, S., and Wang, P. (2023). Broadly neutralizing antibodies to SARS-CoV-2 and other human coronaviruses. Nat. Rev. Immunol. *23*,

189–199. https://doi.org/10.1038/s41577-022-00784-3.

4. Kangro, K., Kurašin, M., Gildemann, K., Sankovski, E., Žusinaite, E., Lello, L.S., Pert, R., Kavak, A., Poikalainen, V., Lepasalu, L., et al. (2022). Bovine colostrum-derived antibodies against SARS-CoV-2 show great potential to serve as prophylactic agents. PLoS One *17*, e0268806. https://doi.org/10.1371/journal.pone.0268806.
5. Halwe, S., Kupke, A., Vanshylla, K., Liberta, F., Gruell, H., Zehner, M., Rohde, C., Krähling, V., Gellhorn Serra, M., Kreer, C., et al. (2021). Intranasal Administration of a Monoclonal Neutralizing Antibody Protects Mice against SARS-CoV-2 Infection. Viruses *13*, 1498. https://doi.org/10.3390/v13081498.
6. Belser, J.A., Eckert, A.M., Huynh, T., Gary, J.M., Ritter, J.M., Tumpey, T.M., and Maines, T.R. (2020). A Guide for the Use of the Ferret Model for Influenza Virus Infection. Am. J. Pathol. *190*, 11–24. https://doi.org/10.1016/j.ajpath.2019.09.017.
7. Enkirch, T., and von Messling, V. (2015). Ferret models of viral pathogenesis. Virology *479–480*, 259–270. https://doi.org/10.1016/j.virol.2015.03.017.
8. Cox, R.M., Wolf, J.D., and Plemper, R.K. (2021). Therapeutically administered ribonucleoside analogue MK-4482/EIDD-2801 blocks SARS-CoV-2 transmission in ferrets. Nat. Microbiol. *6*, 11–18. https://doi.org/10.1038/s41564-020-00835-2.
9. Cox, R.M., Wolf, J.D., Lieber, C.M., Sourimant, J., Lin, M.J., Babusis, D., DuPont, V., Chan, J., Barrett, K.T., Lye, D., et al. (2021). Oral prodrug of remdesivir parent GS-441524 is efficacious against SARS-CoV-2 in ferrets. Nat. Commun. *12*, 6415. https://doi.org/10.1038/s41467-021-26760-4.
10. de Vries, R.D., Rockx, B., Haagmans, B.L., Herfst, S., Koopmans, M.P., and de Swart, R.L. (2021). Animal models of SARS-CoV-2 transmission. Curr. Opin. Virol. *50*, 8–16. https://doi.org/10.1016/j.coviro.2021.06.007.
11. Chatterjee, S., Bhattacharya, M., Nag, S., Dhama, K., and Chakraborty, C. (2023). A Detailed Overview of SARS-CoV-2 Omicron: Its Sub-Variants, Mutations and Pathophysiology, Clinical Characteristics, Immunological Landscape, Immune Escape, and Therapies. Viruses *15*, 167. https://doi.org/10.3390/v15010167.
12. Tao, K., Tzou, P.L., Kosakovsky Pond, S.L., Ioannidis, J.P.A., and Shafer, R.W. (2022). Susceptibility of SARS-CoV-2 Omicron Variants to Therapeutic Monoclonal Antibodies: Systematic Review and Meta-analysis. Microbiol. Spectr. *10*, e0092622. https://doi.org/10.1128/spectrum.00926-22.
13. Planas, D., Saunders, N., Maes, P., Guivel-Benhassine, F., Planchais, C., Buchrieser, J., Bolland, W.H., Porrot, F., Staropoli, I., Lemoine, F., et al. (2022). Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. Nature *602*, 671–675. https://doi.org/10.1038/s41586-021-04389-z.
14. Chiu, N.C., Chi, H., Tai, Y.L., Peng, C.C., Tseng, C.Y., Chen, C.C., Tan, B.F., and Lin, C.Y. (2020). Impact of Wearing Masks, Hand Hygiene, and Social Distancing on Influenza, Enterovirus, and All-Cause Pneumonia During the Coronavirus Pandemic: Retrospective National Epidemiological Surveillance Study. J. Med. Internet Res. *22*, e21257. https://doi.org/10.2196/21257.
15. Linka, K., Peirlinck, M., and Kuhl, E. (2020). The reproduction number of COVID-19 and its correlation with public health interventions. Comput. Mech. *66*, 1035–1050. https://doi.org/10.1007/s00466-020-01880-8.
16. Haldane, V., De Foo, C., Abdalla, S.M., Jung, A.S., Tan, M., Wu, S., Chua, A., Verma, M., Shrestha, P., Singh, S., et al. (2021). Health systems resilience in managing the COVID-19 pandemic: lessons from 28 countries. Nat. Med. *27*, 964–980. https://doi.org/10.1038/s41591-021-01381-y.
17. Wünsch, K., Anastasiou, O.E., Alt, M., Brochhagen, L., Cherneha, M., Thümmler, L., van Baal, L., Madel, R.J., Lindemann, M., Taube, C., et al. (2022). COVID-19 in Elderly, Immunocompromised or Diabetic Patients-From Immune Monitoring to Clinical Management in the Hospital. Viruses *14*, 746. https://doi.org/10.3390/v14040746.
18. Davis, H.E., Mccorkell, L., Vogel, J.M., and Topol, E.J. (2023). Long COVID: major findings, mechanisms and recommendations. Nat. Rev. Microbiol. *21*, 133–146. https://doi.org/10.1038/s41579-022-00846-2.
19. Devi, M.B., Devi, A., Gupta, P.K., and Tripathi, D. (2022). Response of vaccination on community transmission of COVID-19: a dynamical approach. Eur. Phys. J. Spec. Top. *231*, 3749–3765. https://doi.org/10.1140/epjs/s11734-022-00652-0.
20. Bewley, K.R., Coombes, N.S., Gagnon, L., McInroy, L., Baker, N., Shaik, I., St-Jean, J.R., St-Amant, N., Buttigieg, K.R., Humphries, H.E., et al. (2021). Quantification of SARS-CoV-2 neutralizing antibody by wild-type plaque reduction neutralization, microneutralization and pseudotyped virus neutralization assays. Nat. Protoc. *16*, 3114–3140. https://doi.org/10.1038/s41596-021-00536-y.
21. Cox, R.M., Lieber, C.M., Wolf, J.D., Karimi, A., Lieberman, N.A.P., Sticher, Z.M., Roychoudhury, P., Andrews, M.K., Krueger, R.E., Natchus, M.G., et al. (2023). Comparing molnupiravir and nirmatrelvir/ritonavir efficacy and the effects on SARS-CoV-2 transmission in animal models. Nat. Commun. *14*, 4731. https://doi.org/10.1038/s41467-023-40556-8.

## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Bacterial and virus strains | | |
| SARS-CoV -2 WA-1 isolate | BEI Resources | NR-52281 |
| Biological samples | | |
| Immunoglobulin preparation from bovine colostrum, batch 003 | This study | BOSS Ig 003 |
| BioBlock nasal spray | Medihex | https://www.medihex.com/en/products/bioblock/ |
| Nasal spray buffer w/o antibody solution | This study | N/A |
| Chemicals, peptides, and recombinant proteins | | |
| SARS-CoV-2 trimeric spike proteins | This study | N/A |
| ACE2-hFc protein | Icosagen AS | P-308-100 |
| Critical commercial assays | | |
| EZ-LinkTM NHS-PEG4 Biotinylation Kit | ThermoFisher Scientific | 21455 |
| PierceTM High Sensitivity Streptavidin-HRP | ThermoFisher Scientific | 21132 |
| ELISA plates; Maxisorp F8 NuncImmunomodule | ThermoFisher Scientific | 468667 |
| ELISA reader (spectrophotometer) MultiSkan FC | ThermoFisher Scientific | 51119000 |
| Experimental models: Organisms/strains | | |
| Ferret (Mustela putorius furo) | Triple F Farms | NA |
| Software and algorithms | | |
| GraphPad Prism v10.0 | GraphPad Software Inc | https://www.graphpad.com/ |

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Joachim M. Gerhold (joachim.gerhold@icosagen.com).

#### Materials availability

SARS-CoV-2 trimeric S proteins, ACE2-hFc protein used in this study are available either commercially from Icosagen Cell Factory OÜ and Icosagen AS or the lead contact with a completed Materials Transfer Agreement. The viral strain used in this study is commercially available. Biological samples will be made available on request, but we may require payment and/or a completed Materials Transfer Agreement if there is potential for commercial application.

#### Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the reported data in this paper is available from the lead contact upon request.

### EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

#### Animal subjects

This study used female ferrets (Mustela putorius furo), family mustelids, genus mustela, 6-10 months of age. Upon receipt, all ferrets were housed under standard ABSL-1 conditions for an acclimatization period of 72 hours. After 72 hours, ferrets were transferred to ABSL-3 facilities at Georgia State University. All animals were provided standard ferret enrichment and monitored at least once daily throughout the course of

the study. Biological sex of research animals was determined based on examination of external genitalia. Ferret studies were carried out with female animals only. No sex-based analyses were performed because co-housing studies may not be carried out with male ferrets (IACUC protocol A20031), since males are territorial and highly combative when co-housed, resulting in severe injury from fight wounds that would require termination of the study. The study design does not allow for the use of singly housed male ferrets. Sourced animals were randomly assigned to cages by animal support staff blinded for the study. Cages were randomly assigned to study groups, animals weighed, and infected as specified. The investigators were not blinded to group allocation for data collection and analysis for any experiment performed in this study due to size of the research group with clearance for experimentation under high biocontainment conditions required for work with live SARS-CoV-2. Ferrets are large animals that cannot be handled by a single investigator for the experiments reported under ABSL3 conditions and resources available did not allow involvement of additional personnel that would have been required for blinding. All *in vivo* studies with SARS-CoV-2 involving ferrets were approved by the Georgia State Institutional Animal Care and Use Committee under protocol A20031, in compliance with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health guidelines, and the Animal Welfare Act Code of Federal Regulations. All experiments using infectious SARS-CoV-2 strains were approved by the Georgia State Institutional Biosafety Committee under protocol B20016 and performed in BSL-3/ABSL-3 facilities at the Georgia State University.

### Ethics statement

All *in vivo* studies were performed in compliance with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health guidelines, and the Animal Welfare Act Code of Federal Regulations. Experiments with SARS-CoV-2 involving ferrets were approved by the Georgia State Institutional Animal Care and Use Committee under protocol A20031. All experiments using infectious SARS-CoV-2 strains were approved by the Georgia State Institutional Biosafety Committee under protocol B20016 and performed in BSL-3/ABSL-3 facilities at Georgia State University.

## METHOD DETAILS

### Ferret studies

Female ferrets (n=4/group; 6-10 months old; Triple F Farms) were infected intranasally with 1x105 PFU of SARS-CoV-2 WA1. Source ferrets were co-housed with BioBlock or vehicle-treated sentinels at a 1:1 ratio starting 36 hours after infection. Sentinels were intranasally administered BioBlock (0.236 mg/kg) or vehicle (1 ml total dose volume; 500 µL per nare) in 4-hour intervals starting 4 hours prior to the onset of co-housing. Treatment was continued through the 12-hour co-housing period, for a total of five BioBlock administrations. Source and sentinel animals were separated at the end of the 12-hour co-housing period, and clinical signs, bodyweight, and temperature monitored once daily. Nasal lavages were collected from all animals once daily. Source animals were terminated 96 hours after infection and nasal turbinates extracted. Sentinel animals were terminated 84 hours after cohousing start and nasal turbinates extracted. Infectious SARS-CoV-2 titers and viral RNA copy numbers were determined in nasal lavage samples and homogenized nasal turbinates by virus titration on Vero-TMPRSS2 cells and RT-qPCR, respectively.

### SARS-CoV-2 neutralizing antibody ELISA

The efficacy of the Ig preparation in blocking the angiotensin-converting enzyme 2 (ACE2) receptor interaction with the SARS-CoV-2 trimeric spike protein receptor-binding domain (RBD) was determined using an inverted ELISA method.

96-well ELISA microtiter plates (Maxisorp F8 Nunc-Immunomodule, Cat.-no 468667, Thermo Scientific) were coated with SARS-CoV-2 trimeric spike proteins of VOC alpha, delta, or omicron (B.1.1.529, XBB.1.5, BQ.1.1) at 2,5 µlg/ml (100 µl per well) in coating buffer (1x PBS, pH 7.4) for 16 to 24 hours at 4°C. The coating solution was then aspirated, and wells washed 4 times with 300 µl wash-buffer (1 x PBS, 0.05% Tween 20, pH 7,4) using an automated ELISA plate washer. After removal of any residual wash-buffer by tapping plates upside down on dry, clean paper towels, plates were blocked adding 300 µl per well of a blocking buffer (PBS, 1% BPLA, 2% sucrose, pH 7,4). Plates were covered and kept at room temperature for at least one or up to two hours. The blocking buffer was aspirated, and plates were dried at 35°C in a thermostat for 15-16 hours. In case plates were not used immediately, they were vacuum sealed and kept at 4°C until further use. All necessary reagents were equilibrated to room temperature prior to conducting experiments.

Ig preparation samples were two-fold diluted in 12 steps starting from 3200 µg/ml into analysis buffer (1x PBS, 0,5% BPLA, 2% sucrose, 0,1% Proclin 300). 50 µl per well of samples were added to the plates in triplicates. Covered plates were incubated for 20 minutes on an orbital microtiter plate shaker at 450 rpm. Next, 50 µl of enzyme conjugate (1x PBS, 0.5% BPLA, 0.5 µg/ml of biotinylated ACE2-fc, 0.02 µg/ml Streptavidin-HRP (Pierce™ High Sensitivity Streptavidin-HRP, ThermoFisher Scientific, cat.-no. 21132), 0.1% Proclin 300, pH 7.4) was added without touching the samples in the wells. Prior to use, ACE2-fc (Icosagen AS cat. -no P-308-100) was biotinylated using an EZ-Link™ NHS-PEG4 Biotinylation Kit (ThermoFisher Scientific, cat.-no. 21455). Plates were covered and incubated on an orbital microtiter plate shaker at 450 rpm for 30 minutes, then aspirated and washed 4 times with 300 µl wash buffer (as above). 100 µl TMB VII (Biopanda) was added to each well. The plates were covered and incubated for 10 minutes on an orbital microtiter plate shaker at 450 rpm. The coloring reaction was stopped by addition of 50 µl stop solution (0.5 M H2SO4). Plates were briefly shaken, incubated for 1-2 minutes and the optical density (OD) at a wavelength of 450 nm was measured with an ELISA plate reader (spectrophotometer).

OD450 values of measured samples were divided by the mean value of the eight repeated samples of a negative control (analysis buffer, composition as above) to obtain relative OD450 values. Samples with a relative OD450 value of <0.75 were considered sufficient in blocking

ACE2 binding. The threshold of 0.75 was previously determined as the limit of detection (LoD) by comparison of negative sera and sera from COVID19-patients (measurements of level of blank (LoB) and level of detection (LoD) according to the CLSI standard EP17-A2). Data were analyzed, plotted, and graphically summarized using GraphPad Prism 10 (GraphPad Software Inc.).

## QUANTIFICATION AND STATISTICAL ANALYSIS

GraphPad Prism version 10 was used to conduct statistical analyses and generation of XY- and boxplots.

Half-maximal inhibition ($IC_{50}$) was determined through a non-linear regression analysis with a variable slope (four parameters). XY-plots for the immunoglobulin preparation analyses are presented with non-linear fit and standard deviations (SD); n=3 replicates.

XY-plots in Figures 4A and 4B are presented with geometric SD.

Data presented in Figures 4C and 4D were not normally distributed and thus log transformed. The normality test showed Gaussian distribution after transformation. Ordinary one-way ANOVA was then used with Brown-Forsythe test for equal variances. Correction for multiple comparisons was conducted using Šidàk's test. For statistical analyses p values $<0.05$ were considered to be statistically significant. Data in boxplots are presented as mean with SD. n=8 for each treatment (total 32).

Statistical tests used and sample size can be found in the figure legends.