No evidence for enhanced disease with polyclonal SARS-CoV-2 antibody in the ferret model.

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ABSTRACT

Since SARS-CoV-2 emerged in late 2019, it spread from China to the rest of the world. An initial concern was the potential for vaccine- or antibody-enhanced disease (AED) as had been reported with other coronaviruses. To evaluate this, we first developed a ferret model by exposing ferrets to SARS-CoV-2 by either mucosal inoculation (intranasal/oral) or inhalation using a small particle aerosol. Mucosal inoculation caused a mild fever and weight loss that resolved quickly; inoculation via either route resulted in virus shedding detected in the nares, throat, and rectum for 7-10 days post-infection. To evaluate the potential for AED, we then inoculated groups of ferrets intravenously with 0.1, 0.5, or 1 mg/kg doses of a human polyclonal anti-SARS-CoV-2 IgG from hyper-immunized transchromosomic bovines (SAB-185). Twelve hours later, ferrets were challenged by mucosal inoculation with SARS-CoV-2. We found no significant differences in fever, weight loss, or viral shedding after infection between the three antibody groups or the controls. Signs of pathology in the lungs were noted in infected ferrets but no differences were found between control and antibody groups. The results of this study indicate that healthy, young adult ferrets of both sexes are a suitable model of mild COVID-19 and that low doses of specific IgG in SAB-185 are unlikely to enhance the disease caused by SARS-CoV-2.

INTRODUCTION

Coronaviruses (CoV) are positive-stranded RNA viruses of the family *Coronaviridae* in the order *Nidovirales*. Genome size ranges from 26-32 kb. In humans, coronaviruses are mostly associated with the 'common' cold, a mild disease that resolves in 7-10 days. In 2002 a novel coronavirus associated with severe acute respiratory syndrome (SARS-CoV) emerged in Guangdong province, China, with a ~10% case fatality rate. The virus rapidly spread to other countries[1], but the outbreak was contained, resulting in 8,098 known cases and 774 deaths. A second novel coronavirus associated with severe disease emerged in the Middle East in 2012 (Middle East Respiratory Syndrome; MERS); at 35%, MERS-CoV has the highest case fatality rate of the known zoonotic coronaviruses to date but does not readily spread human-to-human[2]. In late 2019, another novel coronavirus (Severe Acute Respiratory Syndrome-CoronaVirus-2, SARS-CoV-2) associated with severe respiratory disease (COVID-19) emerged in Wuhan, China [3, 4]. This new virus had sequence homology with the original SARS virus and was named SARS-CoV-2. Of the severe CoV, SARS-CoV-2 has the lowest case fatality rate but is the most readily transmissible, which accounts for its rapid spread across the globe. Considerable epidemiologic and virologic data support the notion that asymptomatic or pre-symptomatic individuals infected with SARS-CoV-2 can transmit the virus [5-8]. Risk factors for severe or fatal disease include advanced age and co-morbidities such as heart disease, hypertension, obesity or diabetes [9].

One initial area of concern with SARS-CoV-2 was the potential for pre-existing, virus-specific antibodies, such as those induced by vaccination, to enhance disease upon natural infection by the virus. In studies with feline peritonitis virus, kittens that received a vaccinia-vectored vaccine expressing the spike protein succumbed to challenge earlier than control kittens

[10]. Vaccinated kittens had low levels of neutralizing antibody immediately prior to challenge. Passive immunization with antiserum against feline peritonitis virus also caused early death[11, 12]. *In vitro*, virus-specific antiserum increased uptake of the virus by feline macrophages. In another study, mice vaccinated with an inactivated MERS-CoV vaccine had increased eosinophilic lung infiltrates upon challenge [13]. Similarly, mice vaccinated with an alum-adjuvanted SARS-CoV vaccine had enhanced eosinophilia and lung pathology after challenge[14]. Ferrets vaccinated with a modified vaccinia virus Ankara vaccine expressing the spike and nucleocapsid proteins for SARS-CoV had enhanced disease (stronger inflammatory responses & focal necrosis in the liver) when challenged with SARS-CoV [15, 16]. Rhesus macaques vaccinated with an inactivated SARS-CoV vaccine or with vaccinia virus expressing spike protein and challenged with SARS-CoV showed evidence of antibody-enhanced disease[17, 18]. Passive immunization with IgG against SARS-CoV spike abrogated the wound healing process, promoted MCP1 and IL-8 production, and recruited proinflammatory myeloid-lineage cells to the lung. There was cause for concern then that suboptimal antibody response, whether induced by vaccination or passive immunization, could enhance COVID-19[19].

Ferrets are often used as an animal model for studying respiratory viral infections, particularly influenza, respiratory syncytial virus, and SARS-CoV[20-26]. Relevant to both SARS and SARS-CoV-2, the sequence of the ACE2 receptor in ferrets shares considerable homology with that of humans, suggesting that ferrets could be a potential model for COVID-19 [27]. Other groups have indeed shown that healthy, adult ferrets can be infected with SARS-CoV-2 and develop mild disease with considerable virus shedding following infection via intranasal and/or intratracheal inoculation[28-30]. There is also a question of whether SARS-CoV-2 transmission is by contact, droplet, or aerosol, with increasing evidence that aerosol transmission of SARS-

CoV-2 is indeed occurring[31-34]. The virus would be more likely to reach the deep lung if inhaled in a droplet (<5 μm mass median aerodynamic diameter) and this might alter disease course and pathology, as we have previously shown for H5N1[35]. The present study adds to the body of SARS-CoV-2 literature for the ferret model, including different virus isolates, routes of infection, and whether low titers of neutralizing antibody against SARS-CoV-2 spike could enhance disease.

MATERIALS & METHODS

Ethics. The University of Pittsburgh is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All animal work was performed under the standards of the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and according to the Animal Welfare Act guidelines. All animal studies adhered to the principles stated in the Public Health Services Policy on Humane Care and Use of Laboratory Animals. The University of Pittsburgh Institutional Animal Care and Use Committee (IACUC) approved and oversaw the animal protocol for these studies.

Biological safety. All work with SARS-CoV-2 was conducted in the University of Pittsburgh Center for Vaccine Research (CVR) Regional Biocontainment Lab (RBL) under BSL-3 conditions. Respiratory protection for all personnel when handling infectious samples or working with animals was provided by powered air-purifying respirators (PAPRs; Versaflo TR-300; 3M, St. Paul, MN). Liquid and surface disinfection was performed using Peroxigard disinfectant (1:16 dilution), while solid wastes, caging, and animal wastes were steam sterilized in an autoclave.

Ferrets. Eleven adult ferrets of both sexes ranging in weight from 0.8-1.6 kg were purchased from Triple F Farms for use in these studies. Ferrets were neutered or spayed and descented before shipment to the University of Pittsburgh.

Virology. The SARS-CoV-2 isolates used were passage 6 (p6) of the CDC/2019-nCoV/USA_WA1/2020 (WA1) isolate (a gift from Dr. Natalie Thornburg at the Centers for Disease Control in Atlanta) and passage 3 (p3) of the SARS-CoV-2/München-1.1/2020/929 (Munich) isolate (a gift from Drs Christen Drosten & Jan Felix Drexler, Charité –

Universitätsmedizin Berlin) described previously [36]. Virus was titrated by conventional plaque assay on Vero E6 cells (ATCC CRL1586) and titers are expressed as plaque forming units (pfu) and infection was visualized in cells by indirect immunofluorescence, as described [36].

Mucosal inoculation: Ferrets were anesthetized with Isoflurane; once anesthesia was achieved they were inoculated with 1 ml of stock virus given orally, 0.5 ml of stock virus inoculated into each nare, and 0.05 ml of virus inoculated into each eye. Ferrets were returned to their cage and observed until they had fully recovered from anesthesia.

Aerosol exposure. Aerosol exposures were performed using the Aero3G aerosol management platform (Biaera Technologies, Hagerstown, MD). Ferrets were loaded individually into metal exposure cages and transported to a class III biological safety cabinet using a mobile transfer cart. In the class III cabinet, ferrets were placed inside a whole-body exposure chamber and exposed four at a time. Aerosol exposures were 10 minutes in duration. Aerosols were generated using an Aerogen Solo vibrating mesh nebulizer (Aerogen, Chicago, IL) as previously described [37], with a total airflow of 22.0 liters per minute into the chamber. Total exhaust, including sampling, was set to be equal to intake air at the rate of one air change inside the exposure chamber every two minutes. To determine inhaled dose, aerosol sampling was performed during each exposure with an all-glass impinger (AGI) operating at 6 lpm, -6 to -15 psi (AGI; Ace Glass, Vineland, NJ). Particle size was measured once during each exposure at 5 minutes using an Aerodynamic Particle Sizer with a diluter at 1:100 (TSI, Shoreview, MN). Mass median aerodynamic diameter was 1.72 µm with a geometric square deviation of 1.72. After a 5 minute air wash with clean air, the animal was removed from the cabinet and transported back to its cage. Nebulizer and AGI samples were assessed by plaque assay to determine virus concentration and inhaled dose was calculated as the product of aerosol concentration of the

virus and the accumulated volume of inhaled air, determined as the product of the duration of exposure and the animal's minute volume [38].

Telemetry. Each ferret was implanted with a DSI PhysioTel Digital radiotelemetry transmitter (DSI Model No. M00) capable of continuously recording body temperature. Telemetry implants were implanted abdominally and the surgical site closed using skin sutures. During acquisition, data was transmitted from the implant to a TRX-1 receiver mounted in the room connected via a Communications Link Controller (CLC) to a computer running Ponemah v6.5 (DSI) software. Preexposure data collection began at least seven days in advance of infection. Data collected from Ponemah was exported as 15-minute averages into Excel files which were subsequently analyzed in MatLab 2019a as previously described [39, 40]. Using pre-exposure baseline data, an auto-regressive integrated moving average (ARIMA) model was used to forecast body temperature assuming diurnal variation across a 24-hour period. The code is available at https://github.com/ReedLabatPitt/Reed-Lab-Code-Library. Residual temperatures were calculated as actual minus predicted temperatures. Upper and lower limits to determine significant changes were calculated as the product of 3 times the square root of the residual sum of squares from the baseline data.

Clinical Scoring. Clinical signs were recorded twice per day and each animal was given an objective score for weight, temperature, appearance & behavior, and respiratory signs (Supplemental Table I).

Plethysmography. Respiratory function was assessed in ferrets using a whole-body plethysmography chamber and pneumotach connected to a digital preamplifier run by Finepointe v2.8 software (DSI). For purposes of this study, we used a study protocol for chronic obstructive pulmonary disease (COPD) that is defined in Finepointe software. The chamber and pneumotach

were calibrated each day before use. The chamber lid was removed, the ferret was placed in the chamber after which the chamber was sealed. The system was run for 3-5 minutes to acclimate the ferret to the chamber and ensure good data is being collected prior to initiation of data collection which was for 3-5 minutes. Data was analyzed within Finepointe or in Excel & GraphPad.

Quantitative RT-PCR: RNA was extracted from tissues using Trizol reagent and qRT-PCR assays on extracted RNA were performed exactly as previously described [36].

SAB-185 human IgG: Production and purification of the SAB-185 human IgG preparation has been described elsewhere [41-45]. Briefly, transchromosomic bovines were hyperimmunized two times with a DNA plasmid expressing the WA-1 spike (S) protein followed by at least three times with purified recombinant S protein of the same amino acid sequence. Bovines were bled and human IgG purified for use in ferret studies.

Alveolar space analysis: Formaldehyde fixed tissues were paraffin embedded by the University of Pittsburgh McGowan Institute or the Biospecimen Core. Tissue sections were cut to 5 µm and mounted on positively charged glass slides. Standard regressive hematoxylin (Cat # MHS16-500ml) and eosin (Cat # E511-100) staining was used to stain for histopathology. Coverslips were placed on the slides promptly using a 1:1 xylene:toluene mixture as a mounting medium. Using an Olympus Provis microscope, images of stained lungs were captured at 10x for analysis. At least 5 images were captured of the lungs from each ferret. Transmitted light was set at 9.0 and exposure time was kept consistent for all images. The microscope was white balanced, and a blank image was obtained to remove shading. NIS-ElementsAR v5.30.01 was used to remove background shading and scale images to 1.35 µm per pixel 47. Alveolar space analysis was performed on H&E samples post imaging in FIJI v1.53c. Images were converted to 8-bit and

Huang thresholding with dark background was used to distinguish between alveolar spaces and tissues. Regions of interest were selected and alveolar space was assessed using the 'Analyze Particles' function of the FIJI software with size set to 50 μm2 to infinity to exclude nuclei. Summaries and results for each image were saved as Microsoft Excel files.

RESULTS

Comparison of aerosol and mucosal infection with SARS-CoV-2 in ferrets

Prior to evaluating SAB185 for AED potential, we first developed a ferret model of COVID-19 by infecting ferrets with the WA1/2020 isolate. Because transmission of SARS-CoV-2 is primarily airborne, we compared viral inoculation by the traditional 'mucosal' inoculation (IN/oral/ocular) and inhalation of virus via small particle aerosol. Two ferrets were exposed to media alone by either mucosal inoculation or aerosol to serve as mock, uninfected controls (C1 & C2). As expected, there was no fever or substantial (>5%) weight loss in the controls although there is weight loss over time in the controls (Figure 1). Ferrets are prone to lose weight when stressed, and it is possible that the daily monitoring and observations may have been responsible for the weight loss observed. The two mucosally-inoculated ferrets (designated M1 & M2) received a dose of $4x10^6$ pfu; both had a spike in fever within the first 36 hours of infection which returned to normal by 48 hours; there were some small spikes in fever seen at 5-7 dpi and again around 12 dpi. Both mucosally-inoculated ferrets also lost weight fairly rapidly post-infection, reaching 5% around days 2-3 before beginning to rebound. Four ferrets (designated A1-A4) were exposed to WA1/2020 by aerosol with an inhaled dose of $2x10^4$ pfu. Higher doses by aerosol would only have been possible by generating a very large stock of virus and concentrating it via sucrose-gradient purification. Because of concerns regarding adaptation of the virus to cell culture and the significant amount of time and cost required, concentrating the virus was not pursued. No significant fever was seen in the 4 ferrets exposed to WA1/2020 by aerosol and only 1 of the 4 lost >5% of its body weight in the post-infection period.

Viral shedding from mucosal sites was also assessed in these ferrets (Figure 2). In the mucosally-inoculated ferrets, vRNA (viral) was found on oral swabs taken on days 2 and 4 from

both ferrets. Nasal swabs were positive on days 2 and 7 from both mucosal-inoculated ferrets and one of the two on day 10. For both mucosal-inoculated ferrets, vRNA was isolated from rectal swabs only at 2 dpi. Infectious virus was isolated from only M1 at 2 dpi in the oral and nasal cavities and day 4 orally from both M1 and m2 (Supplemental Table 1). Despite the lower dose achieved by aerosol, viral shedding from the oral and nasal cavities was very similar for aerosol-infected ferrets compared to mucosal-inoculated ferrets in terms of genomes/ml and duration. Oral swabs from three of the four aerosol-infected ferrets were positive for vRNA out to day 7; two of the four were also positive for vRNA in the nasal cavity at day 7. Two of the four aerosol-infected ferrets were positive for vRNA from rectal swabs on day 4 and one was positive on day 7. Infectious virus was found in the oral and nasal cavities from all four aerosol-infected ferrets on day 2 but only two were positive on day 4 and one on day 7 (Supplemental Table 1). None of the mucosally-inoculated or aerosol-infected ferrets were positive for infectious virus from the rectal swabs at any timepoint.

Ferrets were euthanized after day 14 post-infection with WA1/2020 and samples collected to evaluate viral load in tissues. No infectious virus was found in any tissues. For the mucosally-inoculated ferrets, vRNA was found sporadically throughout the respiratory tract, intestines, lymph nodes and also the CNS, heart, kidney and bladder (Figure 3). Lower levels of vRNA were found in the aerosol-infected ferrets, and was mostly confined to the respiratory tract.

No evidence for antibody-dependent enhancement of disease with SAB185.

SAB185 is human IgG specific for SARS-CoV-2 that is purified from the plasma of vaccinated transchromosomic cows expressing human immunoglobulin genes. As part of the pre-clinical evaluation of SAB185, we examined whether low doses of SAB185 would induce

enhanced disease in ferrets when challenged with SARS-CoV-2. Based on the model development data, we opted to go with the mucosal inoculation, but with a European isolate that had the D614G isolate (Munich-1.1) instead of WA1/2020. Twelve hours before challenge, ferrets were inoculated IV with SAB185 at doses of 0.1, 0.5, or 1.0 mg/kg. A fourth group was given an irrelevant control IgG against dengue virus. The following morning, all of the ferrets were challenged with $8x10^5$ pfu of Munich-1.1 by mucosal inoculation (IN/oral).

Similar to the WA1/2020 isolate, control ferrets inoculated mucosally with Munich-1.1 spiked a fever 1-2 dpi which subsided within a day although occasional spikes in temperature were seen out to 14 dpi when the study was ended (Figure 4). Similar spikes were seen in all three SAB185 groups but in several of the ferrets from these groups there was a more substantial spike in temperature between 6-10 dpi than was seen in the controls. However, quantitative analysis of the fever response failed to identify a significant difference in the fever response between groups in terms of maximum temperature seen, duration or severity (Supplemental Table 2). Overall, average daily weight loss in all four groups was minimal (less than 5% from baseline) although there was one ferret in the control group that lost nearly 10% of its body weight in the first 3 days after infection but then quickly recovered (Figure 5). One ferret in the 1.0 mg/kg group had a more gradual weight loss over the 14 days post-infection but by study endpoint had only lost 5% of its baseline weight.

No change in viral shedding with SAB185 treatment.

We also examined whether low dose SAB-185 treatment altered the kinetics of viral shedding in the ferrets. Figure 6 shows the levels of vRNA recovered from oral swabs (A), nasal washes (B) and rectal swabs (C) out to 14 days after infection. At all three sites, vRNA titers were higher than those seen in the WA1/2020-infected ferrets and persisted longer. No

significant differences in vRNA levels were seen between the controls and any of the SAB185 groups, suggesting that low-dose SAB185 neither prevented nor enhanced viral shedding/transmission. Infectious virus was also found in the oral swabs and nasal washes from ferrets in all three groups out to day 8 post-infection (Supplemental Table 3). Rectal swabs were negative for infectious virus at all timepoints and in all ferrets.

No evidence for antibody-enhancement of lung pathology

Lungs collected at study endpoint were also evaluated for pathological changes by hematoxylin and eosin staining, with representative images shown in Figure 7. Despite the general lack of overt clinical signs of disease, there was considerable pathology noted in the lungs of all of the infected ferrets. There does not appear to be any enhanced pathology in SAB185-inoculated ferrets at any of the doses tested. Alveolar space analysis of multiple 10x images of lungs from each ferret are shown in Figure 7B. Although the pathology appears worse for the Munich isolate compared to WA1 in the images shown, the alveolar space analysis of multiple images from the lungs of infected ferrets found no significant difference between Munich and the mucosal WA1 group. There appears to be more alveolar space in the ferrets infected with WA1 by aerosol, but this difference was not statistically significant (likely because of the number of animals used and the range of values seen). The apparent difference between aerosol and mucosal could be a function of the virus dose, since the aerosol exposure dose was 100-fold lower than what was given mucosally. Alveolar space analysis found no differences in ferrets inoculated with SAB-185 and challenged with Munich compared to control (no antibody) Munich-infected ferrets. What is notable is that across all the groups, in many of the ferrets the pathology is not uniform within an individual animal, with some areas showing minimal pathology and others showing

considerable pathology. Some regions have 80-90% alveolar space and other regions in the same ferret have less than 20% alveolar space.

DISCUSSION

We have reported here our efforts to develop the ferret as an animal model for COVID-19 and to use that model to evaluate the potential for antibody-enhanced disease. We started with the original U.S. isolate, WA1/2020, and compared 'mucosal' (oral/IN/IT/ocular) inoculation and aerosol. The thought was that inhalation of the virus in a small particle aerosol would be more likely to result in moderate or severe disease than the mucosal inoculation, similar to what we had previously reported with aerosol delivery of H5N1 into cynomolgus macaques which triggered a rapid, acutely lethal infection where prior reports with mucosal inoculation only rarely saw lethal disease[35]. Here, however, inhalation of SARS-CoV-2 did not result in more serious disease in comparison to the mucosal inoculation. It is important to note that the aerosol dose is significantly lower than the mucosal inoculation, which may have altered the outcome. Achieving a higher aerosol dose in the ferrets would have required growing up considerable amounts of SARS-CoV-2 and concentrating the virus. While technically feasible, there were concerns that culture adaptation of the virus could attenuate the virus[36] and that growing up sufficient stocks to support not just model development but future vaccine or drug efficacy studies would require considerable equipment and effort with no guarantee that a higher dose would in fact cause a more severe disease phenotype. For H5N1 viruses, aerosol delivery via small particle aerosol increases the amount of virus reaching the deep lung, where alveolar epithelium expresses the α2,3 sialic acid receptors that the virus binds to. ACE2, which serves as the receptor for SARS-CoV-2, is found throughout the respiratory tract including the bronchial epithelium[46]. In our estimation then, inhalation of SARS-CoV-2 as a small particle aerosol is not necessary for animal studies to understand pathogenesis or evaluate potential vaccines or therapeutics.

While conducting these studies, the D614G mutation in the spike protein emerged in Europe and quickly became the dominant circulating virus around the world. We had obtained a SARS-CoV-2 isolate from Munich which had the D614G mutation. While the WA1/2020 and Munich-1.1 isolates were not tested head-to-head in the same study, the data we obtained in ferrets demonstrated more disease (fever, weight loss) and prolonged viral shedding with Munich-1.1 compared to WA1/2020 even though the dose of Munich-1.1 given was lower. At necropsy, we found evidence for the persistence of viral RNA for WA1/2020 (but not infectious virus) out to at least day 14. This is similar to what we saw in nonhuman primates where viral RNA was isolated from African green monkeys more than a month after infection[47]. In the African green monkeys, we also saw occasional fever spikes that coincided with increased viral RNA at mucosal sites 2-3 weeks after resolution of the original infection. Whether these findings in ferrets and African green monkeys is just remnants of viral RNA or intact virus is not clear but this may warrant further investigation considering reports of chronic COVID-19 in patients with initially mild/moderate disease.

We also partnered with SAb Biotherapeutics to evaluate whether SAB185, a polyclonal humanized antibody product for treatment of SARS-CoV-2 infection, would enhance disease if given at a low dose. Prior published data from other coronaviruses in animal models, including ferrets, have shown evidence for antibody-enhanced disease after vaccination or passive immunization[10-17]. Systemic inflammation, hepatitis, and even death have been observed. Since SARS-CoV-2 produces only mild disease in ferrets[29, 30], we reasoned that if low doses of antibody induced enhanced disease, ferrets might be a good model for making that determination. Our results (fever, weight, viral shedding, pathology), however, would suggest that at least with SAB185 in ferrets at the doses administered, there are no concerns about ADE.

This data was submitted as part of the IND request that was submitted to, and allowed, by the FDA for the ACTIV2 SARS-CoV-2 clinical trial (ClinicalTrials.gov Identifier: NCT04469179).

It was reported that human-derived anti-SARS-CoV-2 antibodies could enhance infection of cells *in vitro* but when these antibodies were infused into mice and macaques they suppressed SARS-CoV-2 replication and did not enhance disease[48]. It is possible that human antibodies do not bind animal FcγRs sufficiently to cause enhanced disease. However, there have been other animal as well as human studies evaluating antibody-based therapies and vaccines and no reports of enhanced disease[48-52]. Based on that and our findings reported here, in addition to the considerable number of human patients that have received monoclonal antibodies or convalescent serum, it seems unlikely that antibody-enhanced disease is a concern for SARS-CoV-2.

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Declaration of Interests

HW, TL, CB, KE, and ES are employees of SAB Biotherapeutics and have financial interests. This work was supported by a contract from SAB Biotherapeutics, Inc., to the University of Pittsburgh (WK).

REFERENCES

1. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med. 2003;348(20):1953-66. PubMed PMID: 12690092.

2. Choudhry H, Bakhrebah MA, Abdulaal WH, Zamzami MA, Baothman OA, Hassan MA, et al. Middle East respiratory syndrome: pathogenesis and therapeutic developments. Future virology. 2019;14(4):237-46. Epub 2020/03/24. doi: 10.2217/fvl-2018-0201. PubMed PMID: 32201499; PubMed Central PMCID: PMCPMC7080179.

3. 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. 2020;579(7798):265-9. Epub 2020/02/06. doi: 10.1038/s41586-020-2008-3. PubMed PMID: 32015508; PubMed Central PMCID: PMCPMC7094943.

4. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-3. Epub 2020/02/06. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507; PubMed Central PMCID: PMCPMC7095418.

5. Wei WE, Li Z, Chiew CJ, Yong SE, Toh MP, Lee VJ. Presymptomatic Transmission of SARS-CoV-2 - Singapore, January 23-March 16, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(14):411-5. Epub 2020/04/10. doi: 10.15585/mmwr.mm6914e1. PubMed PMID:

32271722; PubMed Central PMCID: PMCPMC7147908 Journal Editors form for disclosure of potential conflicts of interest. No potential conflicts of interest were disclosed.

6. Arons MM, Hatfield KM, Reddy SC, Kimball A, James A, Jacobs JR, et al. Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility. N Engl J Med. 2020;382(22):2081-90. Epub 2020/04/25. doi: 10.1056/NEJMoa2008457. PubMed PMID: 32329971.

7. Kimball A, Hatfield KM, Arons M, James A, Taylor J, Spicer K, et al. Asymptomatic and Presymptomatic SARS-CoV-2 Infections in Residents of a Long-Term Care Skilled Nursing Facility - King County, Washington, March 2020. MMWR Morb Mortal Wkly Rep. 2020;69(13):377-81. Epub 2020/04/03. doi: 10.15585/mmwr.mm6913e1. PubMed PMID: 32240128; PubMed Central PMCID: PMCPMC7119514 Journal Editors form for disclosure of potential conflicts of interest. No potential conflicts of interest were disclosed.

8. Furukawa NW, Brooks JT, Sobel J. Evidence Supporting Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 While Presymptomatic or Asymptomatic. Emerg Infect Dis. 2020;26(7). Epub 2020/05/05. doi: 10.3201/eid2607.201595. PubMed PMID: 32364890; PubMed Central PMCID: PMCPMC7323549.

9. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020. Epub 2020/02/08. doi: 10.1001/jama.2020.1585. PubMed PMID: 32031570; PubMed Central PMCID: PMCPMC7042881.

10. Vennema H, de Groot RJ, Harbour DA, Dalderup M, Gruffydd-Jones T, Horzinek MC, et al. Early death after feline infectious peritonitis virus challenge due to recombinant vaccinia

virus immunization. J Virol. 1990;64(3):1407-9. Epub 1990/03/01. doi: 10.1128/JVI.64.3.1407-1409.1990. PubMed PMID: 2154621; PubMed Central PMCID: PMCPMC249267.

11. Stoddart CA, Scott FW. Intrinsic resistance of feline peritoneal macrophages to coronavirus infection correlates with in vivo virulence. J Virol. 1989;63(1):436-40. Epub 1989/01/01. doi: 10.1128/JVI.63.1.436-440.1989. PubMed PMID: 2521188; PubMed Central PMCID: PMCPMC247703.

12. Weiss RC, Scott FW. Antibody-mediated enhancement of disease in feline infectious peritonitis: comparisons with dengue hemorrhagic fever. Comparative immunology, microbiology and infectious diseases. 1981;4(2):175-89. Epub 1981/01/01. doi: 10.1016/0147-9571(81)90003-5. PubMed PMID: 6754243; PubMed Central PMCID: PMCPMC7134169.

13. Agrawal AS, Tao X, Algaissi A, Garron T, Narayanan K, Peng BH, et al. Immunization with inactivated Middle East Respiratory Syndrome coronavirus vaccine leads to lung immunopathology on challenge with live virus. Hum Vaccin Immunother. 2016;12(9):2351-6. Epub 2016/06/09. doi: 10.1080/21645515.2016.1177688. PubMed PMID: 27269431; PubMed Central PMCID: PMCPMC5027702.

14. Bolles M, Deming D, Long K, Agnihothram S, Whitmore A, Ferris M, et al. A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. J Virol. 2011;85(23):12201-15. Epub 2011/09/23. doi: 10.1128/JVI.06048-11. PubMed PMID: 21937658; PubMed Central PMCID: PMCPMC3209347.

15. Weingartl H, Czub M, Czub S, Neufeld J, Marszal P, Gren J, et al. Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets. J Virol. 2004;78(22):12672-6. Epub

2004/10/28. doi: 10.1128/JVI.78.22.12672-12676.2004. PubMed PMID: 15507655; PubMed Central PMCID: PMCPMC525089.

16. Czub M, Weingartl H, Czub S, He R, Cao J. Evaluation of modified vaccinia virus Ankara based recombinant SARS vaccine in ferrets. Vaccine. 2005;23(17-18):2273-9. Epub 2005/03/10. doi: 10.1016/j.vaccine.2005.01.033. PubMed PMID: 15755610; PubMed Central PMCID: PMCPMC7115540.

17. Wang QD, Zhang LF, Kuwahara K, Li L, Liu ZJ, Li TS, et al. Immunodominant SARS Coronavirus Epitopes in Humans Elicited both Enhancing and Neutralizing Effects on Infection in Non-human Primates. Acs Infect Dis. 2016;2(5):361-76. doi: 10.1021/acsinfecdis.6b00006. PubMed PMID: WOS:000376145700007.

18. Liu L, Wei Q, Lin Q, Fang J, Wang H, Kwok H, et al. Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. JCI Insight. 2019;4(4). Epub 2019/03/05. doi: 10.1172/jci.insight.123158. PubMed PMID: 30830861; PubMed Central PMCID: PMCPMC6478436.

19. Iwasaki A, Yang Y. The potential danger of suboptimal antibody responses in COVID-19. Nat Rev Immunol. 2020;20(6):339-41. Epub 2020/04/23. doi: 10.1038/s41577-020-0321-6. PubMed PMID: 32317716; PubMed Central PMCID: PMCPMC7187142.

20. Belser JA, Barclay W, Barr I, Fouchier RAM, Matsuyama R, Nishiura H, et al. Ferrets as Models for Influenza Virus Transmission Studies and Pandemic Risk Assessments. Emerging Infectious Diseases. 2018;24(6):965-71. doi: 10.3201/eid2406.172114. PubMed PMID: WOS:000432430000001.

21. Cross RW, Mire CE, Borisevich V, Geisbert JB, Fenton KA, Geisbert TW. The Domestic Ferret (Mustela putorius furo) as a Lethal Infection Model for 3 Species of Ebolavirus. J Infect

Dis. 2016;214(4):565-9. doi: 10.1093/infdis/jiw209. PubMed PMID: 27354371; PubMed Central PMCID: PMCPMC4957446.

22. Belser JA, Eckert AM, Tumpey TM, Maines TR. Complexities in Ferret Influenza Virus Pathogenesis and Transmission Models. Microbiol Mol Biol Rev. 2016;80(3):733-44. Epub 2016/07/15. doi: 10.1128/MMBR.00022-16. PubMed PMID: 27412880; PubMed Central PMCID: PMCPMC4981671.

23. Johnson-Delaney CA, Orosz SE. Ferret respiratory system: clinical anatomy, physiology, and disease. Vet Clin North Am Exot Anim Pract. 2011;14(2):357-67, vii. doi: 10.1016/j.cvex.2011.03.001. PubMed PMID: 21601818.

24. Lednicky JA, Hamilton SB, Tuttle RS, Sosna WA, Daniels DE, Swayne DE. Ferrets develop fatal influenza after inhaling small particle aerosols of highly pathogenic avian influenza virus A/Vietnam/1203/2004 (H5N1). Virol J. 2010;7:231. Epub 2010/09/17. doi: 1743-422X-7-231 [pii]

10.1186/1743-422X-7-231. PubMed PMID: 20843329.

25. van den Brand JM, Haagmans BL, Leijten L, van Riel D, Martina BE, Osterhaus AD, et al. Pathology of experimental SARS coronavirus infection in cats and ferrets. Vet Pathol. 2008;45(4):551-62. Epub 2008/07/01. doi: 10.1354/vp.45-4-551. PubMed PMID: 18587105.

26. Chu YK, Ali GD, Jia F, Li Q, Kelvin D, Couch RC, et al. The SARS-CoV ferret model in an infection-challenge study. Virology. 2008;374(1):151-63. Epub 2008/02/01. doi: 10.1016/j.virol.2007.12.032. PubMed PMID: 18234270; PubMed Central PMCID: PMCPMC2831213.

27. 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(36):22311-22. Epub 2020/08/23. doi: 10.1073/pnas.2010146117. PubMed PMID: 32826334.

28. Park SJ, Yu KM, Kim YI, Kim SM, Kim EH, Kim SG, et al. Antiviral Efficacies of FDA-Approved Drugs against SARS-CoV-2 Infection in Ferrets. mBio. 2020;11(3). Epub 2020/05/24. doi: 10.1128/mBio.01114-20. PubMed PMID: 32444382; PubMed Central PMCID: PMCPMC7244896.

29. Kim YI, Kim SG, Kim SM, Kim EH, Park SJ, Yu KM, et al. Infection and Rapid Transmission of SARS-CoV-2 in Ferrets. Cell host & microbe. 2020;27(5):704-9 e2. Epub 2020/04/08. doi: 10.1016/j.chom.2020.03.023. PubMed PMID: 32259477; PubMed Central PMCID: PMCPMC7144857.

30. Shi J, Wen Z, Zhong G, Yang H, Wang C, Huang B, et al. Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2. Science. 2020;368(6494):1016-20. Epub 2020/04/10. doi: 10.1126/science.abb7015. PubMed PMID: 32269068; PubMed Central PMCID: PMCPMC7164390.

31. Zhang RY, Li YX, Zhang AL, Wang Y, Molina MJ. Identifying airborne transmission as the dominant route for the spread of COVID-19. Proceedings of the National Academy of Sciences of the United States of America. 2020;117(26):14857-63. doi: 10.1073/pnas.2009637117. PubMed PMID: WOS:000548160900013.

32. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. N Engl J Med. 2020;382(16):1564-7. Epub 2020/03/18. doi: 10.1056/NEJMc2004973. PubMed PMID: 32182409; PubMed Central PMCID: PMCPMC7121658.

33. Liu Y, Ning Z, Chen Y, Guo M, Liu YL, Gali NK, et al. Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. Nature. 2020. doi: 10.1038/s41586-020-2271-3. PubMed PMID: WOS:000541051000001.

34. Lednicky JA, Lauzardo M, Hugh Fan Z, Jutla A, Tilly TB, Gangwar M, et al. Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2020. Epub 2020/09/20. doi: 10.1016/j.ijid.2020.09.025. PubMed PMID: 32949774.

35. Wonderlich ER, Swan ZD, Bissel SJ, Hartman AL, Carney JP, O'Malley KJ, et al. Widespread Virus Replication in Alveoli Drives Acute Respiratory Distress Syndrome in Aerosolized H5N1 Influenza Infection of Macaques. J Immunol. 2017;198(4):1616-26. doi: 10.4049/jimmunol.1601770. PubMed PMID: 28062701.

36. Klimstra WB, Tilston-Lunel NL, Nambulli S, Boslett J, McMillen CM, Gilliland T, et al. SARS-CoV-2 growth, furin-cleavage-site adaptation and neutralization using serum from acutely infected hospitalized COVID-19 patients. The Journal of general virology. 2020. Epub 2020/08/22. doi: 10.1099/jgv.0.001481. PubMed PMID: 32821033.

37. Bowling JD, O'Malley KJ, Klimstra WB, Hartman AL, Reed DS. A Vibrating Mesh Nebulizer as an Alternative to the Collison Three-Jet Nebulizer for Infectious Disease Aerobiology. Appl Environ Microbiol. 2019;85(17). Epub 2019/06/30. doi: 10.1128/AEM.00747-19. PubMed PMID: 31253680; PubMed Central PMCID: PMCPMC6696971.

38. Roy CJ, Pitt MLM. Infectious Disease Aerobiology: Aerosol Challenge Methods. In: Swearengen JR, editor. Biodefense Research Methodology and Animal Models. Second ed. Boca Raton, FL: CRC Press; 2012. p. 65-80.

39. Ma H, Lundy JD, Cottle EL, O'Malley KJ, Trichel AM, Klimstra WB, et al. Applications of minimally invasive multimodal telemetry for continuous monitoring of brain function and intracranial pressure in macaques with acute viral encephalitis. PLoS One. 2020;15(6):e0232381. Epub 2020/06/26. doi: 10.1371/journal.pone.0232381. PubMed PMID: 32584818.

40. Reed DS, Lackemeyer, M.G., Garza, N.L., Norris, S., Gamble, S., Sullivan, L.J., Lind, C.M., Raymond, J.L. Severe encephalitis in cynomolgus macaques exposed to aerosolized eastern equine encephalitis virus. Journal of Infectious Diseases. 2007;196:441-50.

41. Taiwo BO, Chew KW, Moser C, Wohl DA, Daar ES, Li JZ, et al. Phase 2 safety and antiviral activity of SAB-185, a novel polyclonal antibody therapy for non-hospitalized adults with COVID-19. J Infect Dis. 2023. Epub 2023/01/21. doi: 10.1093/infdis/jiad013. PubMed PMID: 36661240.

42. Saied AA, Nascimento MSL, do Nascimento Rangel AH, Skowron K, Grudlewska-Buda K, Dhama K, et al. Transchromosomic bovines-derived broadly neutralizing antibodies as potent biotherapeutics to counter important emerging viral pathogens with a special focus on SARS-CoV-2, MERS-CoV, Ebola, Zika, HIV-1, and influenza A virus. Journal of medical virology. 2022;94(10):4599-610. Epub 2022/06/03. doi: 10.1002/jmv.27907. PubMed PMID: 35655326; PubMed Central PMCID: PMCPMC9347534.

43. Tang J, Grubbs G, Lee Y, Wu H, Luke TC, Egland KA, et al. Increased Antibody Avidity and Cross-Neutralization of Severe Acute Respiratory Syndrome Coronavirus 2 Variants by Hyperimmunized Transchromosomic Bovine-Derived Human Immunoglobulins for Treatment of Coronavirus Disease 2019. J Infect Dis. 2022;226(4):655-63. Epub 2022/02/03. doi: 10.1093/infdis/jiac031. PubMed PMID: 35106573; PubMed Central PMCID: PMCPMC8903330.

44. Gilliland T, Liu Y, Li R, Dunn M, Cottle E, Terada Y, et al. Protection of human ACE2 transgenic Syrian hamsters from SARS CoV-2 variants by human polyclonal IgG from hyper-immunized transchromosomic bovines. bioRxiv. 2021. Epub 2021/08/04. doi: 10.1101/2021.07.26.453840. PubMed PMID: 34341790; PubMed Central PMCID: PMCPMC8328057.

45. Liu Z, Wu H, Egland KA, Gilliland TC, Dunn MD, Luke TC, et al. Human immunoglobulin from transchromosomic bovines hyperimmunized with SARS-CoV-2 spike antigen efficiently neutralizes viral variants. Hum Vaccin Immunother. 2022;18(2):1940652. Epub 2021/07/07. doi: 10.1080/21645515.2021.1940652. PubMed PMID: 34228597; PubMed Central PMCID: PMCPMC8290372.

46. Ziegler CGK, Allon SJ, Nyquist SK, Mbano IM, Miao VN, Tzouanas CN, et al. SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. Cell. 2020;181(5):1016-+. doi: 10.1016/j.cell.2020.04.035. PubMed PMID: WOS:000537399500010.

47. Hartman AL, Nambulli S, McMillen CM, White AG, Tilston-Lunel NL, Albe JR, et al. SARS-CoV-2 infection of African green monkeys results in mild respiratory disease discernible by PET/CT imaging and shedding of infectious virus from both respiratory and gastrointestinal tracts. PLoS Pathog. 2020;16(9):e1008903. Epub 2020/09/19. doi: 10.1371/journal.ppat.1008903. PubMed PMID: 32946524.

48. Li D, Edwards RJ, Manne K, Martinez DR, Schafer A, Alam SM, et al. In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and neutralizing antibodies. Cell. 2021. Epub 2021/07/10. doi: 10.1016/j.cell.2021.06.021. PubMed PMID: 34242577; PubMed Central PMCID: PMCPMC8232969.

49. Gupta A, Gonzalez-Rojas Y, Juarez E, Crespo Casal M, Moya J, Rodrigues Falci D, et al. Effect of Sotrovimab on Hospitalization or Death Among High-risk Patients With Mild to Moderate COVID-19: A Randomized Clinical Trial. JAMA. 2022;327(13):1236-46. Epub 2022/03/15. doi: 10.1001/jama.2022.2832. PubMed PMID: 35285853; PubMed Central PMCID: PMCPMC8922199.

50. Baum A, Ajithdoss D, Copin R, Zhou A, Lanza K, Negron N, et al. REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. Science. 2020;370(6520):1110-5. Epub 2020/10/11. doi: 10.1126/science.abe2402. PubMed PMID: 33037066; PubMed Central PMCID: PMCPMC7857396.

51. Lempp FA, Soriaga LB, Montiel-Ruiz M, Benigni F, Noack J, Park YJ, et al. Lectins enhance SARS-CoV-2 infection and influence neutralizing antibodies. Nature. 2021;598(7880):342-7. Epub 2021/09/01. doi: 10.1038/s41586-021-03925-1. PubMed PMID: 34464958.

52. Rogers TF, Zhao F, Huang D, Beutler N, Burns A, He WT, et al. Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. Science. 2020;369(6506):956-63. Epub 2020/06/17. doi: 10.1126/science.abc7520. PubMed PMID: 32540903; PubMed Central PMCID: PMCPMC7299280.

FIGURE LEGENDS

Figure 1. Mild fever and weight loss in ferrets infected with WA1/2020 either mucosally or by aerosol. Graphs show residual 6-hour median body temperatures (difference between actual and predicted temperatures) (A-C) for individual ferrets in each group as determined from ARIMA modeling. Graphs in D-F show percent change in body weight (D-F) in control mock-infected ferrets (A, D), mucosally-inoculated ferrets (B, E) and aerosol-infected ferrets (C, F).

Figure 2. Viral shedding from ferrets after mucosal or aerosol infection with WA1/2020. Data shown is in genomes/ml determined from qRT-PCR for ferrets infected with WA1/2020 via mucosal (top) and aerosol (bottom) delivery of virus from A, B) oral swabs, C,D) nasal swabs, and E, F) rectal swabs.

Figure 3. Viral RNA is predominantly found in the respiratory tract and gastrointestinal system of SARS-CoV-2-infected ferrets. Results shown are in log10 genomes/gm of tissue for the two mucosal-infected ferrets and four aerosol-infected ferrets from tissues collected at study endpoint.

Figure 4. No difference in fever response to SARS-CoV-2 challenge in SAB185-inoculated ferrets. Graphs show residual 6-hour median body temperatures (difference between actual and predicted temperatures) (A-D) for individual ferrets in each group as determined from ARIMA modeling. A) Control ferrets, B) 0.1 mg/kg SAB-185, C) 0.5 mg/kg SAB-185, D) 1.0 mg./kg SAB-185.

Figure 5. Minimal weight loss after infection with SARS-CoV-2 in SAB-185 inoculated ferrets, regardless of dose. Graphs shown daily percent change in weight from baseline, pre-challenge weights.

Figure 6. Viral shedding from mucosal sites is not affected in SAB-185 inoculated ferrets after Munich challenge. Graphs show average and standard deviation of viral titer as determined by qRT-PCR at mucosal sites over time for each group as determined from A) oral, B) nasal, and C) rectal swabs after challenge.

Figure 7. Low dose SAB185 does not affect lung pathology of ferrets infected with SARS-CoV-2. A) hematoxylin and eosin staining of representative lung sections from each group, 2x magnification. B) superplot of alveoloar space analysis from lung sections imaged at 10x. Multiple 10x images of the lung from individual ferrets within a group are shown as the same color without borders; the mean for that ferret is the colored symbol with the black border. The black line and error bars are the median value with the interquartile range for that group.

Supplemental Table 1. Plaque assay results for infectious virus in mucosal samples recovered from ferrets after infection with WA1/2020

Route	Ferret	*D2*			*D4*			*D7*			*D14*		
		oral	nasal	rectal	oral	nasal	rectal	oral	nasal	rectal	oral	nasal	rectal
Mucosal	M1	+	+	–	+	–	–	–	–	–	–	–	–
	M2	–	–	–	+	–	–	–	–	–	–	–	–
Aerosol	A1	+	+	–	–	+	–	–	–	–	–	–	–
	A2	+	–	–	–	–	–	–	–	–	–	–	–
	A3	–	+	–	–	–	–	–	–	–	–	–	–
	A4	+	+	–	+	–	–	–	+	–	–	–	–

Supplemental Table 2. Fever data for SAB-185-inoculated and control groups

Group	Ferret	ΔTmax[a]	Fever Duration[b]	Fever-Hours[c]	Ave Elevation[d]
1*	F67-20	1.62	84.00	69.30	0.83
	F69-20	1.10	48.25	30.88	0.64
	F71-20	1.49	57.25	44.06	0.77
	Average	**1.40**	**63.17**	**48.08**	**0.74**
0.5*	F65-20	1.00	34.50	22.62	0.66
	F72-20	1.37	40.50	27.64	0.68
	F66-20	1.23	40.25	28.58	0.71
	Average	**1.20**	**38.42**	**26.28**	**0.68**
0.1*	F63-20	1.09	6.75	4.33	0.64
	F68-20	1.93	44.50	41.15	0.92
	F70-20	1.11	20.25	13.81	0.68
	Average	**1.38**	**23.83**	**19.77**	**0.75**
Control	F61-20	1.49	21.75	16.09	0.74
	F62-20	1.65	30.25	26.55	0.88
	F64-20	1.58	28.00	23.65	0.84
	Average	**1.57**	**26.67**	**22.10**	**0.82**

*dose of SAB-185, in mg/kg

[a] maximum residual difference in temperature, in degrees Celsius

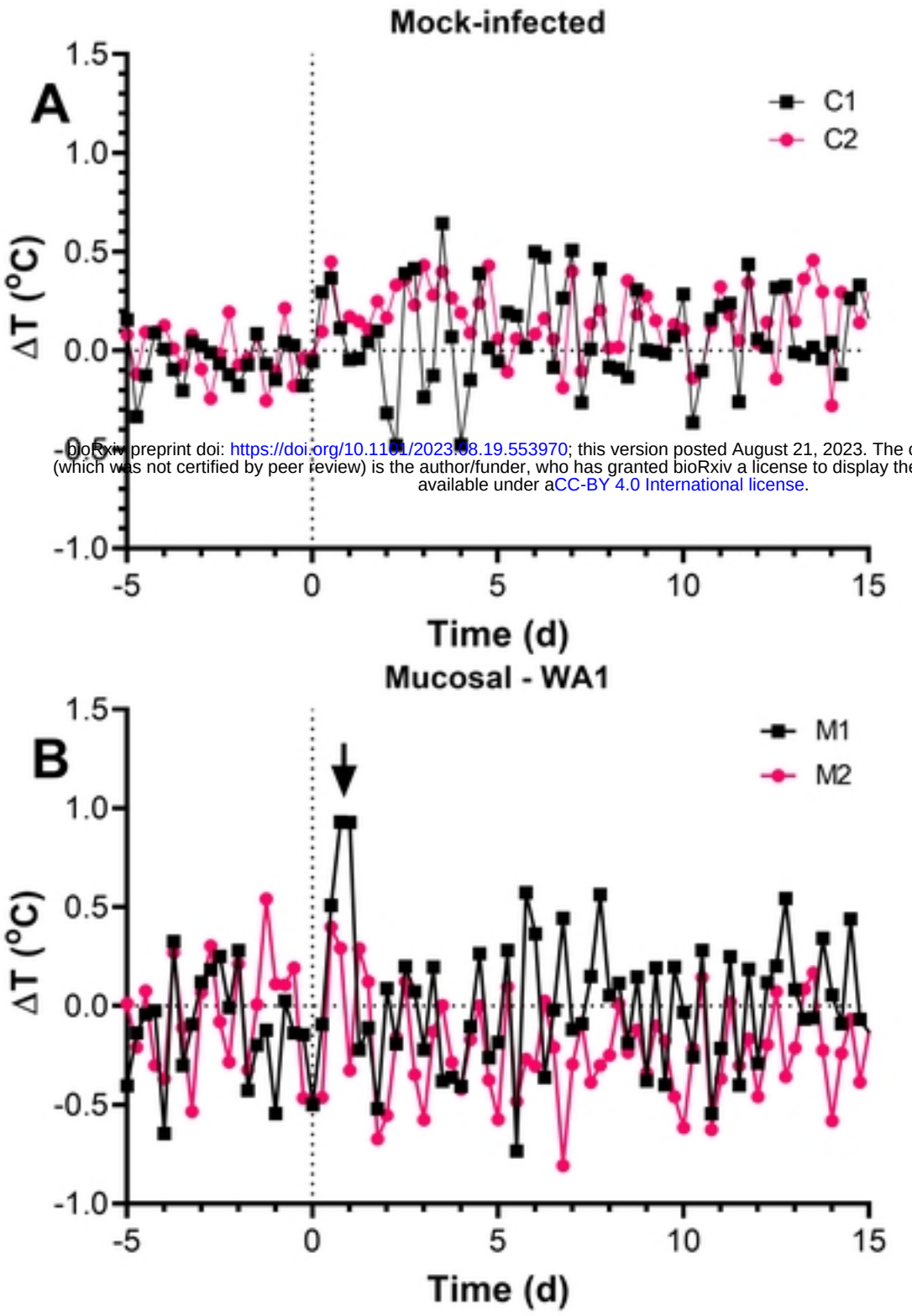
b fever duration in hours

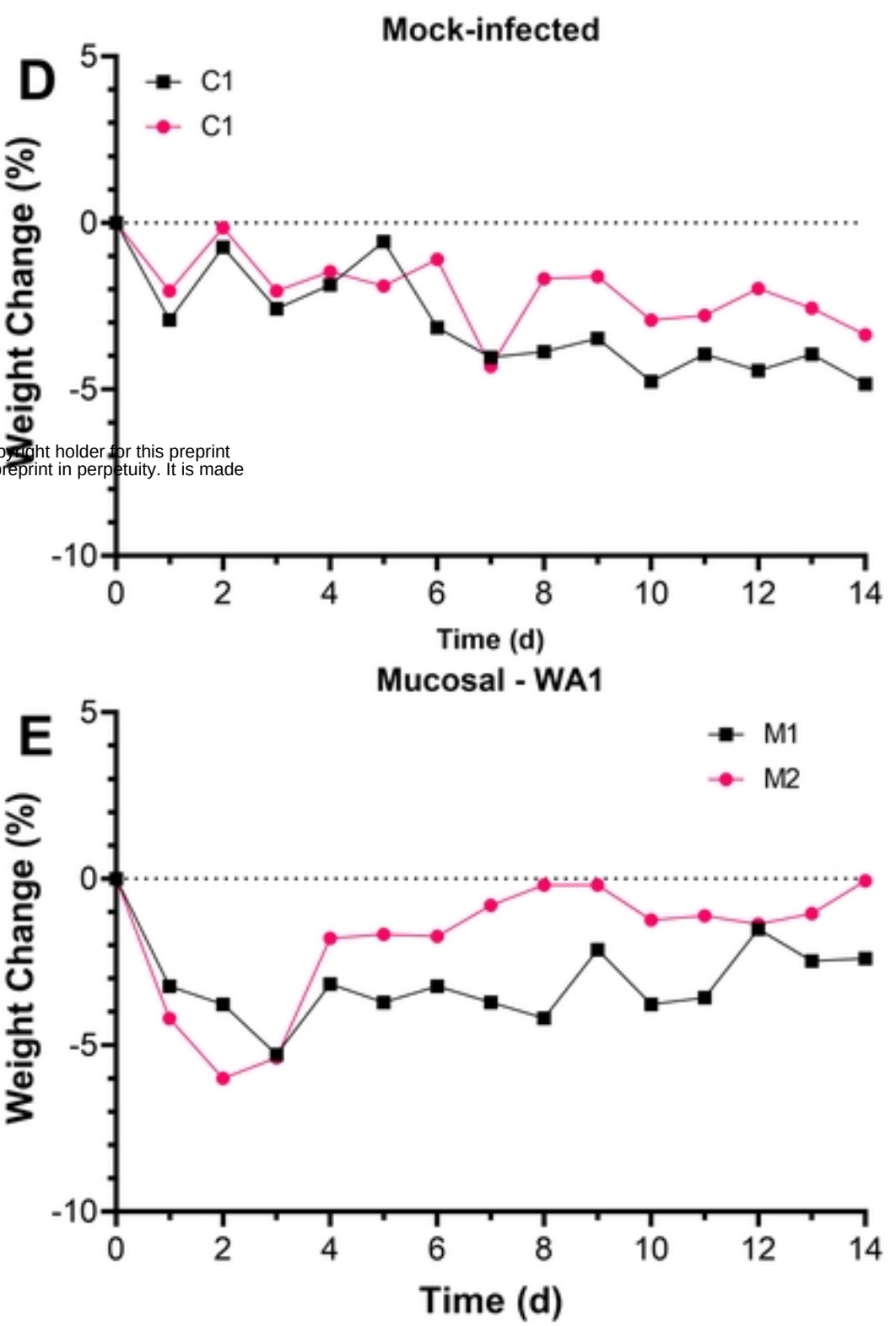
[c] sum of significant residual elevations in body temperature, divided by 4 to convert to fever-hours

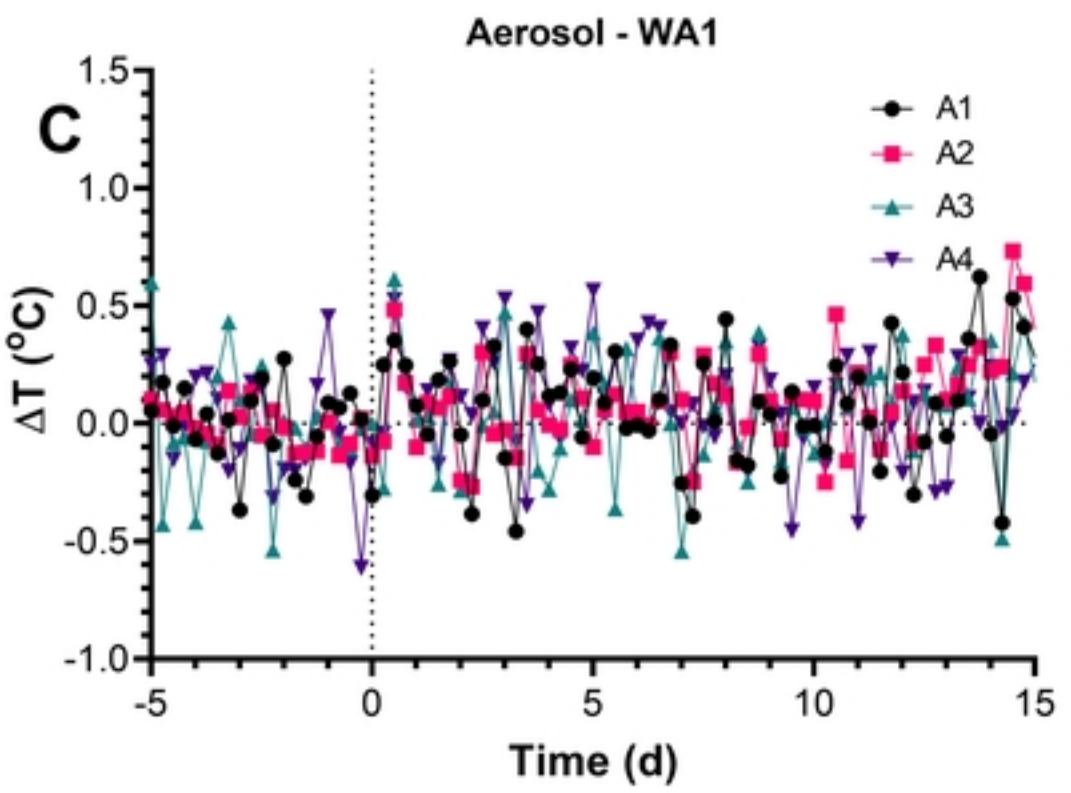
[d] average residual difference in temperature, in degrees Celsius

Supplemental Table 3. Plaque assay results for infectious virus in mucosal samples from SAB-185 inoculated and control ferrets after challenge with Munich.

		D2			*D4*			*D6*			*D8*			*D14*		
Group	Ferret	oral	nasal	rectal	oral	nasal	rectal	oral	nasal	rectal	oral	nasal	rectal	oral	nasal	rectal
Control	F61	+	-	-	+	+	-	+	+	-	+	—	—	—	—	—
	F62	-	+	-	+	+	-	+	-	-	-	—	—	—	—	—
	F64	+	-	-	+	-	-	+	-	-	-	—	—	—	—	—
0.1 mg/kg	F63	+	+	-	+	+	-	-	?+	-	-	—	—	—	—	—
	F68	-	+	-	+	-	-	+	+	-	?+	—	—	—	—	—
	F70	-	+	-	+	+	-	+	+	-	-	—	—	—	—	—
0.5 mg/kg	F65	+	+	-	-	+	-	+	+	-	-	—	—	—	—	—
	F66	-	+	-	+	+	-	+	+	-	-	—	—	—	—	—
	F72	+	+	-	+	+	-	-	-	-	-	—	—	—	—	—
1.0 mg/kg	F67	?+	-	-	-	-	-	-	-	-	-	—	—	—	—	—
	F69	+	+	-	+	x	-	+	+	?+	-	—	—	—	—	—
	F71	+	+	-	+	+	-	+	+	?+	-	—	—	—	—	—







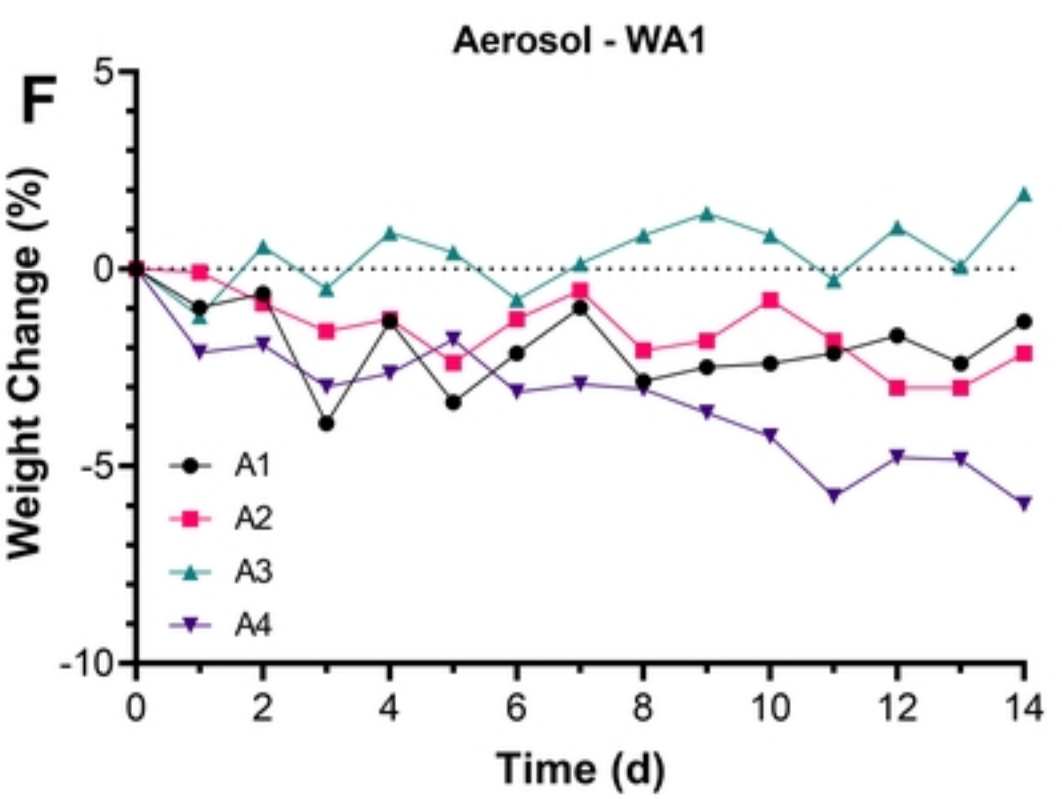


Figure 1

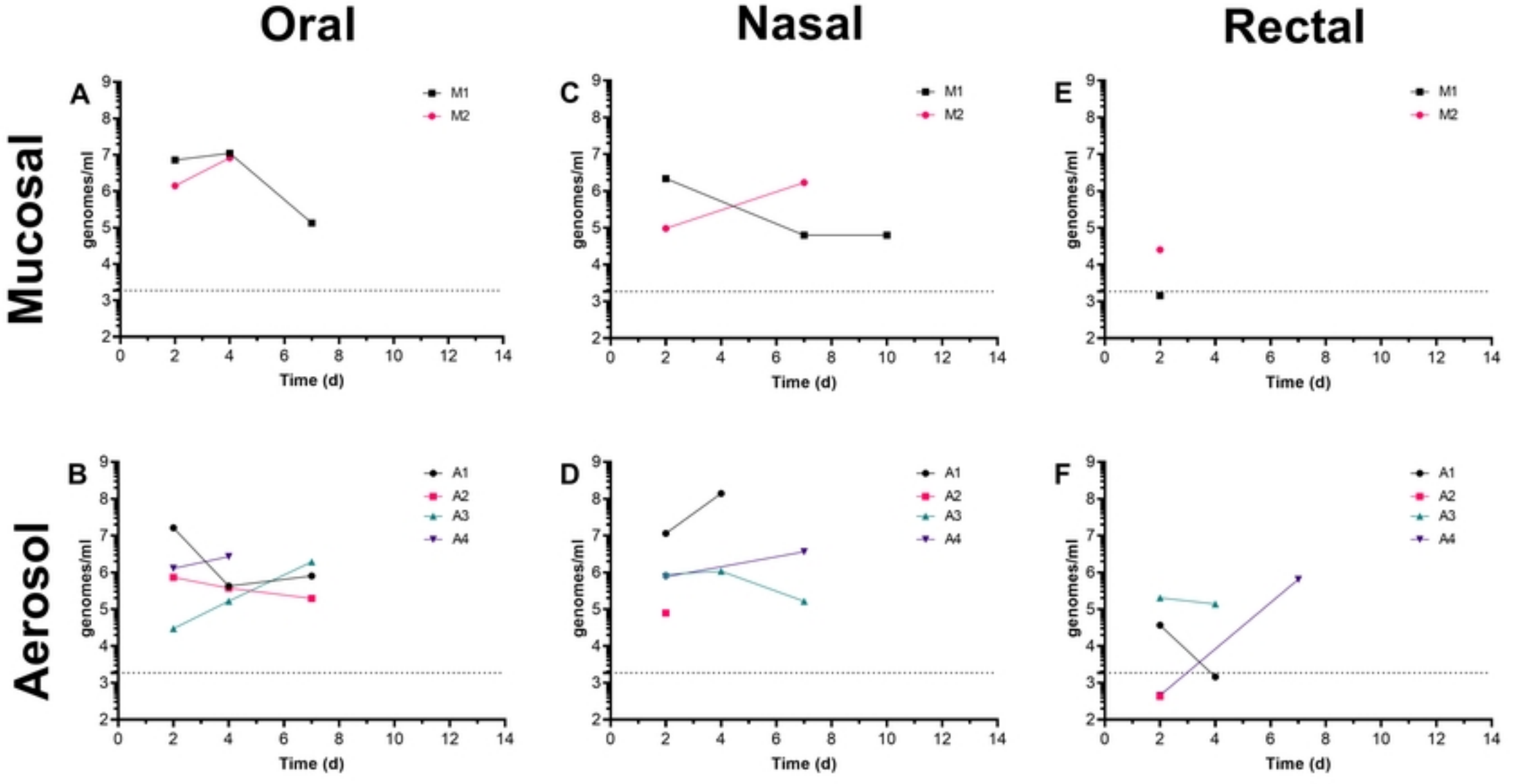

Oral
Nasal
Rectal
Mucosal
Aerosol
A
B
C
D
E
F
M1
M2
A1
A2
A3
A4
genomes/ml
Time (d)

Figure 2

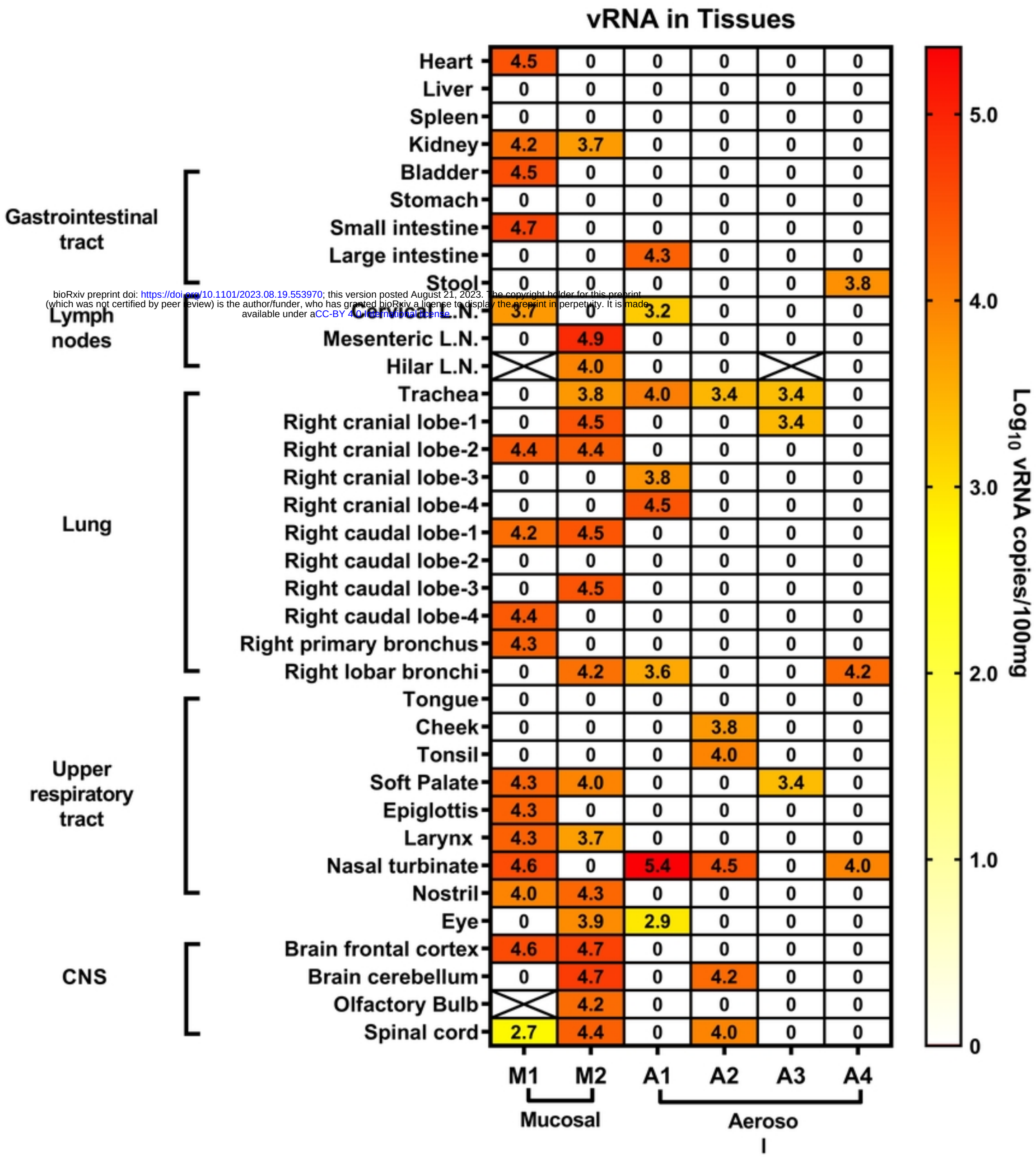
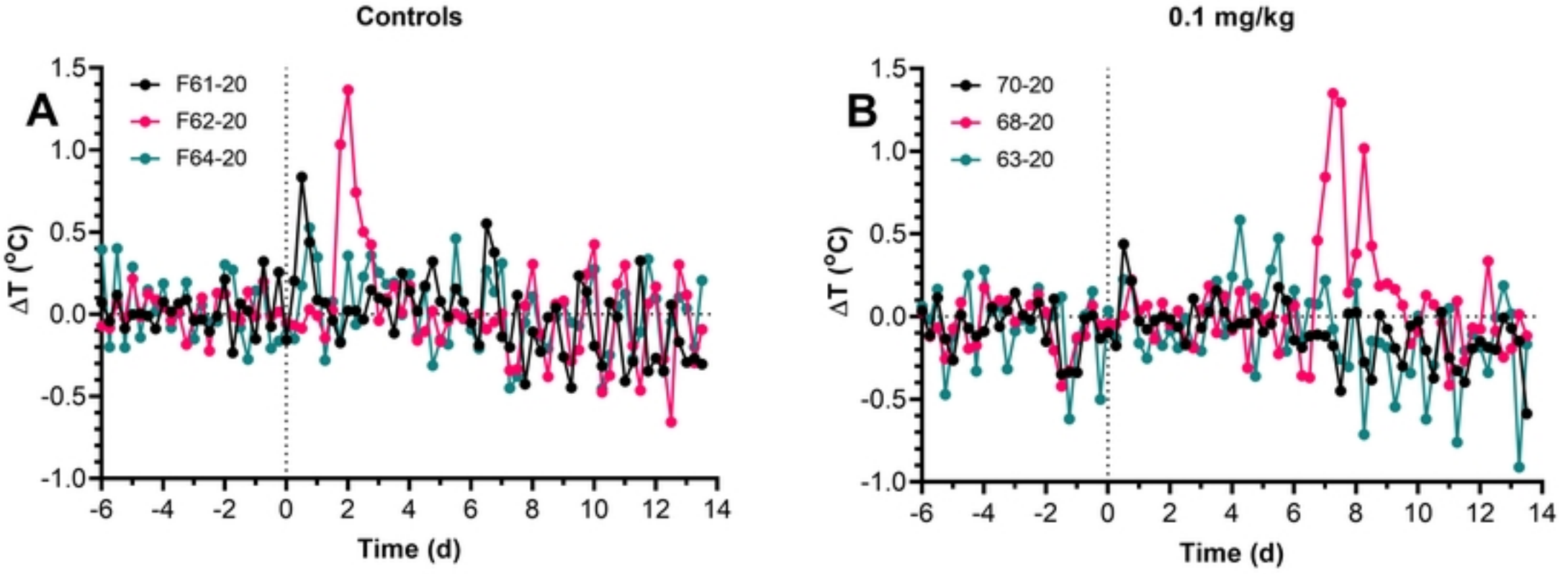


Figure 3


Controls
A
F61-20
F62-20
F64-20
ΔT (°C)
Time (d)
0.1 mg/kg
B
70-20
68-20
63-20
ΔT (°C)
Time (d)
0.5 mg/kg
C
65-20
72-20
66-20
ΔT (°C)
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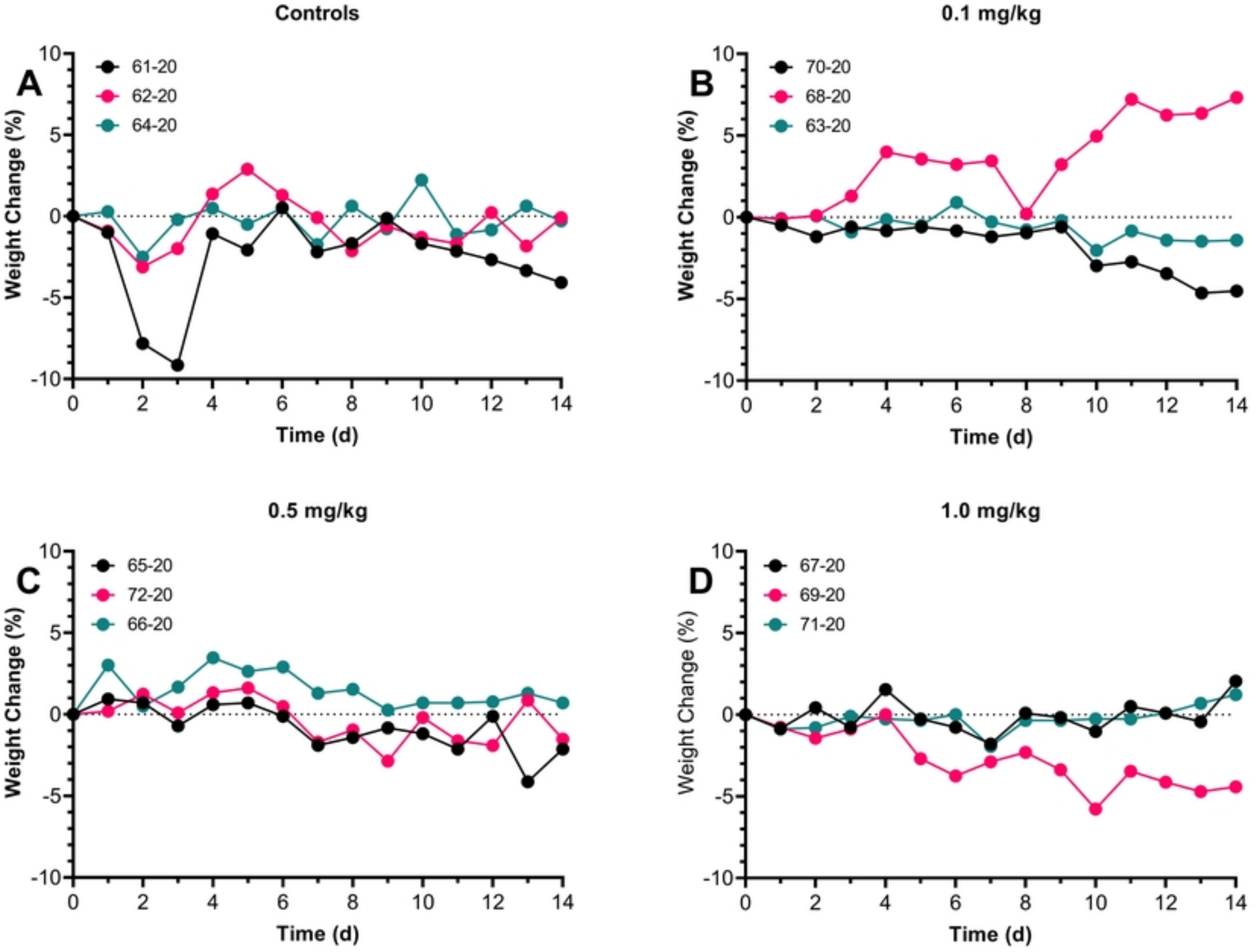
1.0 mg/kg
D
67-20
69-20
71-20
ΔT (°C)
Time (d)

Figure 4


Controls
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61-20
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B
70-20
68-20
63-20
Weight Change (%)
Time (d)
0.5 mg/kg
C
65-20
72-20
66-20
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Time (d)
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D
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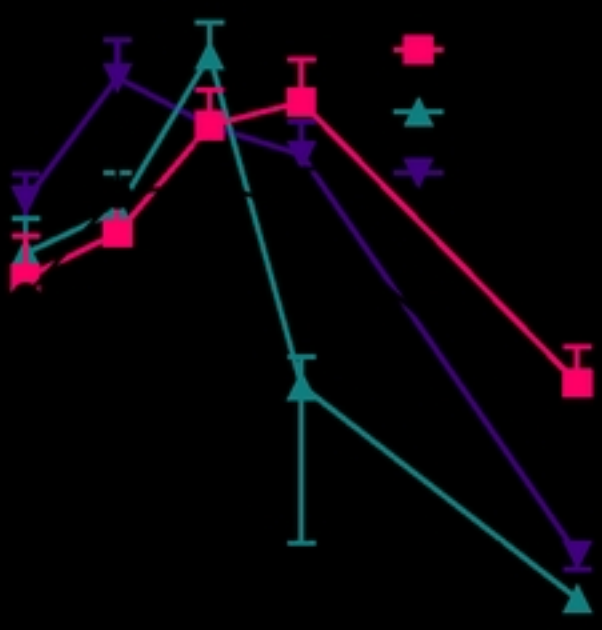
69-20
71-20
Weight Change (%)
Time (d)

Figure 5



https://doi.org/10.1101/2023.08.19.553970

Figure 6

A
100
0
Mucosal WA1
Aerosol WA1
1 mg/kg SAB185
0.5 mg/kg SAB185
0.1 mg/kg SAB185
Munich

Figure 7