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14. ABSTRACT

COVID-19 is a global public health emergency caused by the SARS-CoV-2 virus. Food safety is a major consideration during a pandemic. Food products, such as produce, may become inadvertently contaminated during harvest, production and subsequent sales to consumers in the United States. Moreover, a fecal-oral transmission risk may exist due to the prolonged excretion of SARS-CoV-2 from the gastrointestinal tract: several reports have identified the presence of high levels of SARS-CoV-2 RNA in feces, although infectious virus has not yet been recovered. We do not currently know the risk posed by oral exposure to the SARS-CoV-2 virus. The SARS-CoV-2 virus infects human cells by binding to the ACE2 receptor. The hamster has a similar ACE2 gene to the human and therefore is a good candidate for an animal model of COVID-19. Our first study of oral inoculation of hamsters indicated shedding of viral RNA in the feces for at least 7 days with the UK variant. The objective of this study was to examine the disease state that develops in the hamster following oral infection with SARS-CoV-2. Two variants of the SARS-CoV-2 were tested. WA-1 strain showed little indication of infection, but this particular virus stock was known to have a mutation in the furan cleavage site, which

15. SUBJECT TERMS

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The science you expect.
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SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Final Report

FOR

U.S. Food and Drug Administration

Office of Counterterrorism and Emerging Threats (OCET)

Robert W. Fisher, Ph.D.
Senior Advisor for CBRN and Pandemic Influenza
10903 New Hampshire Avenue
Silver Spring, Maryland 20993

MRIGlobal Project No. 110890.01.208.06.01.03

February 1, 2023

Preface

This final report was prepared at MRIGlobal for the work performed under MRIGlobal Project No. 110980.01.208.06.01.03, for the study entitled, "SARS-CoV-2 Infection Via the Oral Exposure In Hamsters." Robert Fisher, Ph.D. was the sponsor representative for the study.

The study was initiated on August 2, 2021 and completed August 16, 2021. The study was managed by Kristina Eichhorst as study coordinator. She was assisted by Kelsey Burenheide, Emily Chushuk, Amelia Hunziger, JW Grace, Matt Konda, Hana Mayfield, John Moore, Luca Popescu, D.V.M., Ph.D., and Julie Roseman. The original Study Director, Carl Gelhaus, Ph.D. is no longer with MRIGlobal and was replaced by Gene Olinger, Ph.D. after study completion.

Although it was not required for this study to be performed in accordance with FDA GLP (21 *CFR* Part 58) regulations, MRIGlobal ensures that quality processes were in place for the performance of the study, and the work procedures, the data, and the final report were reviewed for quality. All raw data, including original study records, data sheets, work sheets, and copies of reports are stored at MRIGlobal.

MRIGLOBAL

[redacted]

Gene Olinger, Ph.D.
Study Director

Approved by:

[redacted]

Phillip H. Beske, Ph.D., D.A.B.T.
Director, Medical Research Portfolio

February 1, 2023

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Section 1.
Abstract

COVID-19 is a global public health emergency caused by the SARS-CoV-2 virus. Food safety is a major consideration during a pandemic. Food products, such as produce, may become inadvertently contaminated during harvest, production and subsequent sales to consumers in the United States. Moreover, a fecal-oral transmission risk may exist due to the prolonged excretion of SARS-CoV-2 from the gastrointestinal tract: several reports have identified the presence of high levels of SARS-CoV-2 RNA in feces, although infectious virus has not yet been recovered. We do not currently know the risk posed by oral exposure to the SARS-CoV-2 virus. The SARS-CoV-2 virus infects human cells by binding to the ACE2 receptor. The hamster has a similar ACE2 gene to the human and therefore is a good candidate for an animal model of COVID-19. Our first study of oral inoculation of hamsters indicated shedding of viral RNA in the feces for at least 7 days with the UK variant. The objective of this study was to examine the disease state that develops in the hamster following oral infection with SARS-CoV-2. Two variants of the SARS-CoV-2 were tested. WA-1 strain showed little indication of infection, but this particular virus stock was known to have a mutation in the furan cleavage site, which may have impacted the ability of the virus to infect. The UK strain established an infection, with modest signs of infection (rough coat and squinty eyes), a possible trend toward weight loss, and substantial amounts of SARS-CoV-2 RNA in feces, rectal swabs, and nasal washes, demonstrating successful infection. $TCID_{50}$ was negative for all samples, so it is unclear if infectious viral particles were being shed via feces.

Section 2.
Objective and Justification

The objective of this study was to examine the disease state that develops in the hamster following oral infection with SARS-CoV-2. Two variants of the SARS-CoV-2 were tested.

Section 3.
Sponsor, Testing Laboratory, and Personnel Responsibilities

3.1 Sponsor

Office of Counterterrorism and Emerging Threats (OCET)
Senior Advisor for CBRN and Pandemic Influenza
10903 New Hampshire Ave., Silver Spring, MD 20993

3.2 Sponsor's Representative

Robert Fisher, Ph.D.
Phone: (301) 796-8518

3.3 Testing Laboratory

MRIGlobal
425 Dr. Martin Luther King Jr. Blvd.
Kansas City, MO 64110
Phone: (816) 753-7600
Fax: (816) 753-8823

3.3.1 Testing Site

While MRIGlobal oversaw the testing, actual performance of the experiments was executed at:

Laboratory for Infectious Disease Research (LIDR)
1020 East Campus Loop
University of MO
Columbia, MO 65211

3.4 Personnel Responsibilities/Study Director

Original Study Director
H. Carl Gelhaus, Ph.D.
Phone: (816) 753-7600, ext. 5068
email: cgelhaus@mriglobal.org

Current Study Director
Gene Olinger, Ph.D.
Phone: (240) 361-4009
email: golinger@mriglobal.org

Section 4.
Materials and Methods

4.1 Challenge agent

Two isolates of the SARS-CoV-2 were used. The first isolate challenge material was SARS-CoV-2 isolate USA-WA1/2020 from BEI Resources. This virus has been passed through Vero cells once to obtain a master virus bank and the master virus bank was passed through Vero cells to obtain a working virus bank. The working virus bank was tested in this study.

Table 1. Challenge Article – WA1

Name:	SARS-CoV-2
Virus Classification:	*Coronaviridae*
Strain/Isolate:	USA-WA1/2020
Lot Number	20210214HCG
$TCID_{50}$/mL	1.67×10^{6}
Copy number/mL	1.1×10^{10}
Supplier	MRIGlobal (derived from BEI Resources)
Storage Conditions:	≤ -70°C

The second isolate challenge material was SARS-CoV-2 isolate USA/CA_CDC_5574/2020 from BEI Resources. The PANGO lineage for this isolate is the same as the UK variant (B.1.1.7). This virus has been passed through TMPRSS2 cells once to obtain a single virus bank, which was tested in this study.

Table 2. Challenge Article – UK

Name:	SARS-CoV-2
Virus Classification:	*Coronaviridae*
Strain/Isolate:	USA/CA_CDC_5574/2020
Lot Number	TS 04FEB21
$TCID_{50}$/mL	1.1×10^{7}
Copy number/mL	9.0×10^{10}
Supplier	MRIGlobal (derived from BEI Resources)
Storage Conditions:	≤ -70°C

4.2 Test Animals

4.2.1 Species/Strain

Syrian Golden Hamsters

Species: *Mesocricetus auratus*
Strain: Outbred

4.2.2 Source

Envigo
Indianapolis, IN

4.2.3 Sex and Number

7 male and 7 female

4.2.4 Age

The hamsters used for this study were born on May 21, 2021. The animals were received on August 2, 2021.

4.2.1 Criteria

Hamsters were free from obvious clinical signs of ill health or malformation or any other condition that would, in the judgment of a veterinarian or the Study Director, interfere with the conduct of the study. Animals were replaced prior to challenge, if necessary.

4.2.2 Animal Identification

The hamsters were identified with ear tags with individual ID numbers.

The cages bore cards detailing the project number, animal identification numbers (ear tags), dose group (cohort) number, identification and concentration of test article received, route, and the date dosing occurred. The identification of each animal was verified at dosing, and upon death.

4.3 Animal Care

General procedures for animal care and housing were followed in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council, National Academic Press, 2011), the Animal Welfare Act (US Public Law 89-544 and all subsequent amendments), and the Public Health Service, "Policy on Humane Care and Use of Laboratory Animals" (revised 2015).

4.3.1 Diet

All animals were provided with Envigo Teklad Food (Product Code 2018C Lot 062921MA) *ad libitum.* Municipal water was provided *ad libitum*.

4.3.2 Housing

Hamsters have been shown to transmit SARS-CoV-2 animal to animal. Therefore, all animals will be individually housed in polycarbonate Tecniplast caging (Tecniplast, Phoenixville, PA) throughout the acclimation/quarantine period and transferred to metabolic caging prior to infection. The metabolic caging will allow for collection of feces on a daily basis. The hamsters will be housed in environmentally controlled rooms. The rooms will be maintained at a temperature of 68.0° to 79.0°F and a relative humidity of 50% ± 20% with a 12-h light/dark cycle per day. Room temperature, humidity, and light cycles will be monitored by the OceaView® monitoring system 24-h/day (see SOP MRI-1170, "Temperature and Humidity Monitoring in Animal Rooms"). Environmental enrichment will be provided as described in SOP MRI-1505, "Performance and Documentation of Animal Husbandry and Animal Room Maintenance."

4.4 Experimental Design

Hamsters were weighed during the quarantine and acclimation period, prior to the initiation of this protocol.

Hamsters were exposed to virus on Study Day 0 via oral gavage. Hamsters were individually housed in metabolic cages. Six (3 male and 3 female) hamsters were infected with SARS-CoV-2 WA1/2020. An additional six (3 male and 3 female) hamsters were infected with SARS-CoV-2 UK B.1.1.7 variant. In addition, one male and one female were orally dosed with sterile DPBS media as negative controls.

Every day post-infection, a feces pellet was collected from metabolic cages. Hamsters were anesthetized dailyfor the collection of rectal swabs and body weights. Rectal swabs were collected using BD Universal Viral Transport Collection Kits according to manufacturer's instructions. Animals were monitored for 7 days post infection. On Study Days 2 and 4, hamsters had the nasal passages washed with phosphate buffered saline (PBS). Feces were weighed at collection and frozen at < -60°C until analysis. Nasal washes and rectal swabs in transport media were stored at < -60°C. All nasal washes, feces homogenates, and rectal swab solutions were enumerated by PCR and $TCID_{50}$ assays. Animals were euthanized and carcasses disposed without examination.

Table 3. Study Design

Group	Size (N)	Route of Viral Administration	Isolate	Study Duration	Endpoints
1	6 (3/sex)	Oral up to 1 mL; likely 1e6 $TCID_{50}$/mL Challenge Concentration	Washington (Pass 7)	7 Day	Fecal Collection, Rectal Swabs, and BW Daily and Nasal Wash on SD 2 and 4
2	6 (3/sex)		B.117 UK (Pass 4)		
3	2 (1/sex)	Oral (control – DPBS)	Control –DPBS		

4.5 Anesthesia

Table 4. Inhaled Anesthetic

Drug	Dose (e.g., mg/kg or mL/kg)	Dose Volume (mL)	Route	Frequency
Isoflurane	1%-5%	NA	Inhaled	Daily for 8 days (Study Day 0 through 7)

Use of sedation and/or anesthesia for performing various procedures in hamsters, included oral gavage challenge, nasal washes, rectal swabs, body weight measurements and prior to euthanasia.

4.5.1 Randomization

Animals were weighed and allocated to challenge groups according to the study design in Table 3. Animals were allocated to challenge groups using a randomization schedule stratified by sex and body weight, such that average body weights within each treatment group and sex will be approximately equivalent. Randomization was conducted in Microsoft Excel and the order is shown in Appendix B.

4.5.2 Oral Gavage

On the day of challenge (Study Day 0), undiluted virus will be thawed and maintained on wet ice for challenge. The viruses were diluted in DPBS. The $TCID_{50}$ of the challenge materials was determined to be 2.37×10^6 $TCID_{50}$/mL for WA-1 and $3.16 \times 10^5 \times 10^5$ $TCID_{50}$/mL for UK.

Oral gavage challenge was conducted according to SOP MRI-1835, "Intravenous, Subcutaneous, Intramuscular, Intraperitoneal, Dermal, Intravenous, Intratracheal, and Oral Dose Administration to Rodents," while the animals were anesthetized with isoflurane. The challenge dose was inoculated by oral gavage, approximately 0.8 mL using a syringe with a gavage needle. Final doses were 1.90×10^6 $TCID_{50}$ for WA-1 and 2.53×10^5 $TCID_{50}$ for UK.

4.5.3 Clinical Observations

General health observations were performed once daily during the quarantine and acclimation period and prior to the study start. Hamsters were observed at least twice daily after challenge from Study Day 0 through Study Day 7.

All observations were conducted in accordance with SOP MRI-1528, "Procedure for Observation of Animals." Observations were made without opening cages, to prevent transmission of SARS-CoV-2 infection from one animal to another.

4.5.4 Feces Collection

Hamsters were individually housed in metabolic cages with a feces collection tube. Once per day, the feces were collected into an individually labelled, pre-weighed tube for each hamster on each day. One aliquot was made for $TCID_{50}$, a second aliquot for PCR, and excess feces was collected into a third tube (the excess feces tube will not be weighed). The PCR and $TCID_{50}$ tubes were weighed after the feces was added to determine the mass of feces in each tube. Feces in tubes was stored at $\leq$ -60°C until analyzed.

4.5.5 Body Weights

Hamsters were weighed during quarantine. Body weights were measured daily beginning on Study Day 0 through Study Day 7. Body weights were collected in accordance with SOP MRI-2020, "Weighing Rodents and Non-rodents."

4.5.6 Rectal Swabs

Beginning on Study Day 1 through Study Day 7, hamsters were anesthetized and one swab from the viral transport kit was inserted into the rectum of one hamster and rotated briefly. The swab was placed in the viral transport media and stored at 2°-8°C until they were analyzed by $TCID_{50}$ and PCR.

4.5.7 Nasal Washes

Viral infection was also assessed by obtaining nasal washes on Study Days 2 and 4. Sterile DPBS was administered intranasally to isoflurane anesthetized hamsters. Volumes for nasal wash administration were approximately 600 µL, administered 300 µL/nostril via a 1 mL syringe attached to a 22-gauge intravenous catheter tip. The expelled fluid was collected in a sterile petri dish and aliquoted into two cryogenic storage tubes at approximately 200 µL each. Both aliquots were frozen at < -60°C until they are analyzed by $TCID_{50}$ and PCR (one aliquot for each assay). The nasal wash procedure followed the draft SOP entitled, "Nasal Wash in Small Animal Models."

4.5.8 $TCID_{50}$

One aliquot of each feces sample, each nasal wash, and rectal swab was used to assess presence of infectious virus, in accordance with SOP MRI-8701, "50% Tissue Culture Infectious Dose ($TCID_{50}$) Titration of Biological Safety Level (BSL)-3 Coronaviruses."

4.5.9 PCR

One aliquot of each feces sample, each nasal wash, and rectal swab was used to assess presence of infectious virus in a real time quantitative PCR (RT-qPCR) assay. RT-qPCR assays was performed in accordance with SOP MRI-9770, "Nucleic Acid Quantification using Real-Time Polymerase Chain Reaction (PCR)."

4.5.10 Euthanasia

There were no early euthanasia events or animals found dead on study. Hamsters were euthanized on Study Day 7 via isoflurane anesthesia followed by bilateral pneumothorax in accordance with SOP MRI-2330, "Euthanasia of Rodents." No necropsy was performed.

4.5.11 Statistical Analysis of Data

4.5.11.1 Body Weight

Changes in body weight were visualized by graphing subject-specific trajectories over time, with study groups identified using different color lines. Descriptive statistics (mean ± SD, median, minimum, and maximum) for body weight were generated. Body weight on Study Day 0 was considered the baseline weight. The study was not designed for statistical model analysis of body weights.

4.5.11.2 Fecal, Nasal Wash, and Rectal Swab Viral Burden

Descriptive statistics (mean ± SD, median, minimum, and maximum) for infectious viral load in feces, nasal washes, and rectal swabs were generated for both and PCR results for each study group. No descriptive statistics or graphs were prepared for $TCID_{50}$, as all values were negative. Graphs were generated that show the mean ± standard error of these variables as a function of sacrifice day, treatment (treated vs. placebo). Results were visually inspected by the Study Director to assess the likelihood of statistically significant differences.

4.5.11.3 Clinical Observations

The number and proportion of animals showing clinical signs and symptoms of infection (e.g., increased respiration, lethargy) in each study group on each study day were tabulated.

Section 5.
Results

5.1 Clinical Observations

General health observations were performed at least twice daily after challenge from Study Day 0 through Study Day 7. All observations were conducted in accordance with SOP MRI-1528, "Procedure for Observation of Animals." Observations were made without opening cages to prevent transmission of SARS-CoV-2 infection from one animal to another. Relevant group average signs are shown in Table 5. All males, including control males, and many females displayed rough coat, and this was often slight. This is a common finding and cannot be attributed specifically to the SARS-CoV-2. There was single occurrence of squinty eye (right eye, animal 6586, Female, Group 2, UK strain, Study Day 7) and slight chomping (animal 6595, Female, Group 1, WA-1 strain, Study Day 7). As these were not consistently present, they are considered unrelated to the SARS-CoV-2 infection. Clinical observation data can be found in Appendix C.

Table 5. Group Clinical Signs

		Males					
		Group 3 untreated		Group 1 WA-1		Group 2 UK	
Animals examined	N	1		3		3	
Skin/Fur	N	1	c	3		3	
Rough coat	%	100		100		100	
		Females					
		Group 3 untreated		Group 1 WA-1		Group 2 UK	
Animals examined	N	1		3		3	
Eyes	N	0	c	0		1	
squinty eye	%	0		0		33.3	
Skin/Fur	N	0	c	2		3	
Rough coat	%	0		66.7		100	
Other	N	0	c	1		0	
Slight Chomping	%	0		33.3		0	

5.2 Body Weights

Hamsters were weighed during quarantine. Body weights were measured daily beginning on Study Day 0 through Study Day 7. Body weights were collected in accordance with SOP MRI-2020, "Weighing Rodents and Non-rodents." Body weights are shown in Figure 1. There was no evidence of change in body weight in the WA-1 strain. While there is no clear evidence that UK strain infected hamsters had body weight loss, two male animals (animals 6593 and 6597) did have decreasing body weights after infection. While this is not definitive, it is worth considering in future studies. Complete body weight data are in Appendix D.

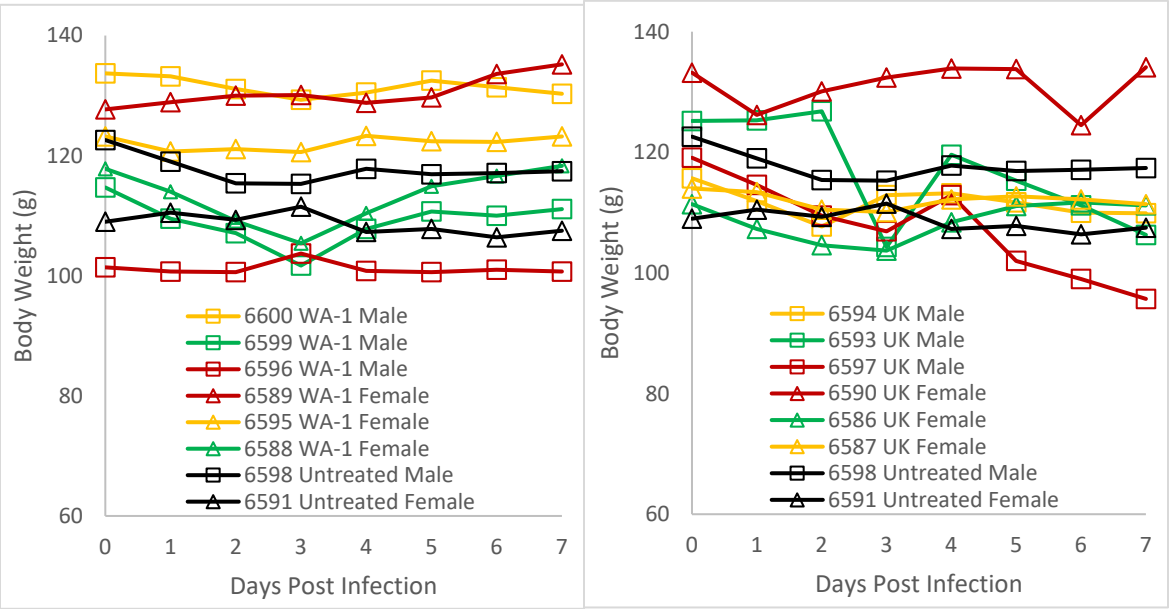

Figure 1. Body Weights.
Left panel is WA-1 and right panel is UK. Individual animals are identified by ear tag number and sex.

5.3 $TCID_{50}$

One aliquot of each feces sample, each nasal wash, and rectal swab was used to assess presence of infectious virus, in accordance with SOP MRI-8701, "50% Tissue Culture Infectious Dose ($TCID_{50}$) Titration of Biological Safety Level (BSL)-3 Coronaviruses." All fecal samples and rectal swabs were negative for all specimens in all groups at all time points. For the nasal wash specimens on Study Days 2 and 4, only male UK infected hamsters were positive, with two of three positive for virus on Study Day 2 and all three positive on Study Day 4 (Figure 2). All $TCID_{50}$ results are shown in Appendix E. As described and discussed below, the appearance of nasal titers only in the UK strain infected hamsters is consistent with the appearance of PCR titers predominantly in the UK strain infected hamsters. This suggest that infections were only stably established in UK strain exposed hamsters.

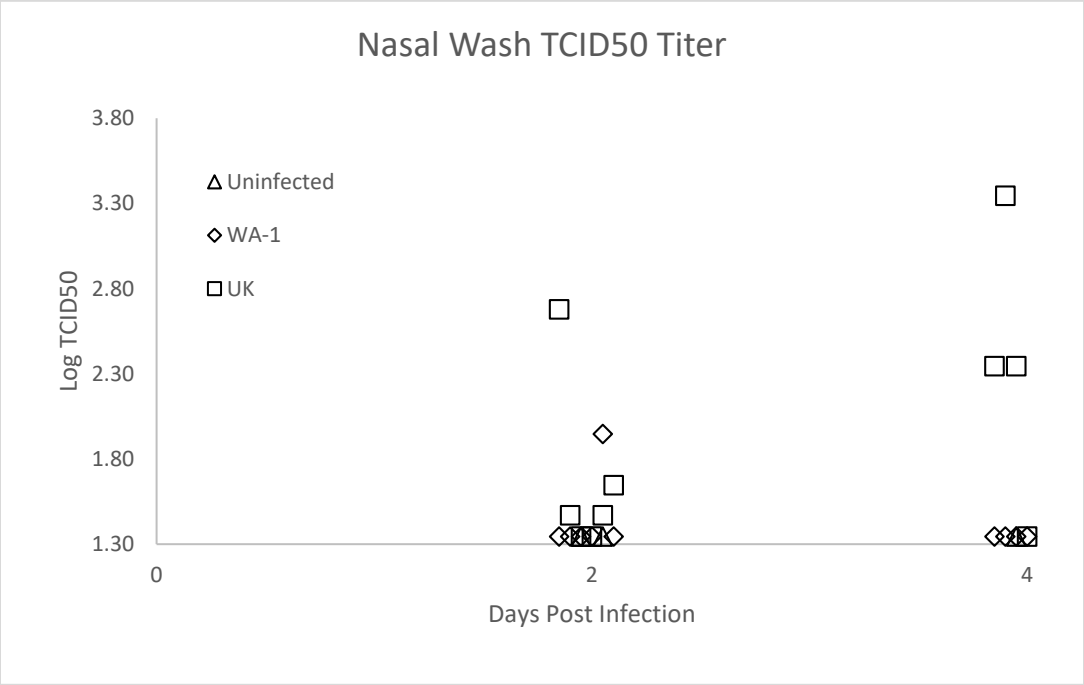

Figure 2. Nasal Wash Titers by $TCID_{50}$.
Uninfected, UK infected and WA-1 infected hamsters had nasal passages washed with DPBS and titers were determined on Vero cells. Most animals showed no infection, although some UK infected hamsters had nasal titers by $TCID_{50}$. Limit of quantiation for this assay was 1.35 log $TCID_{50}$

5.4 PCR

One aliquot of each fecal sample, each nasal wash, and rectal swab was used to assess presence of infectious virus in a real time quantitative PCR (RT-qPCR) assay. For fecal results, there was clear detection of UK variant RNA in most animals every day post-infection. While there were a range of titers, values reached as high as 1×10^8 copies/g feces (Figure 3). The shedding remained constant for this group until the end of the study at Day 7 post-infection. Similar results were observed for rectal swabs, although Day 1 post-infection had only one animal with a very low positive result (6597 Group 2, UK, male, 8.6×10^2 copies/mL of viral transport media on Day 1 post-infection). Rectal swabs tended to produce 1-2 logs lower number of copies/mL of viral transport media relative to copies/g feces, although the range was broad. Feces sampling is likely to give better results in future studies. For the WA-1 variant, fecal samples had SARS-CoV-2 on the first day after infection, but only sporadically at low levels after that. The control samples also had sporadically low levels, indicating some low level of cross contamination at some point. There were no positives in either the WA-1 or control rectal swab samples.

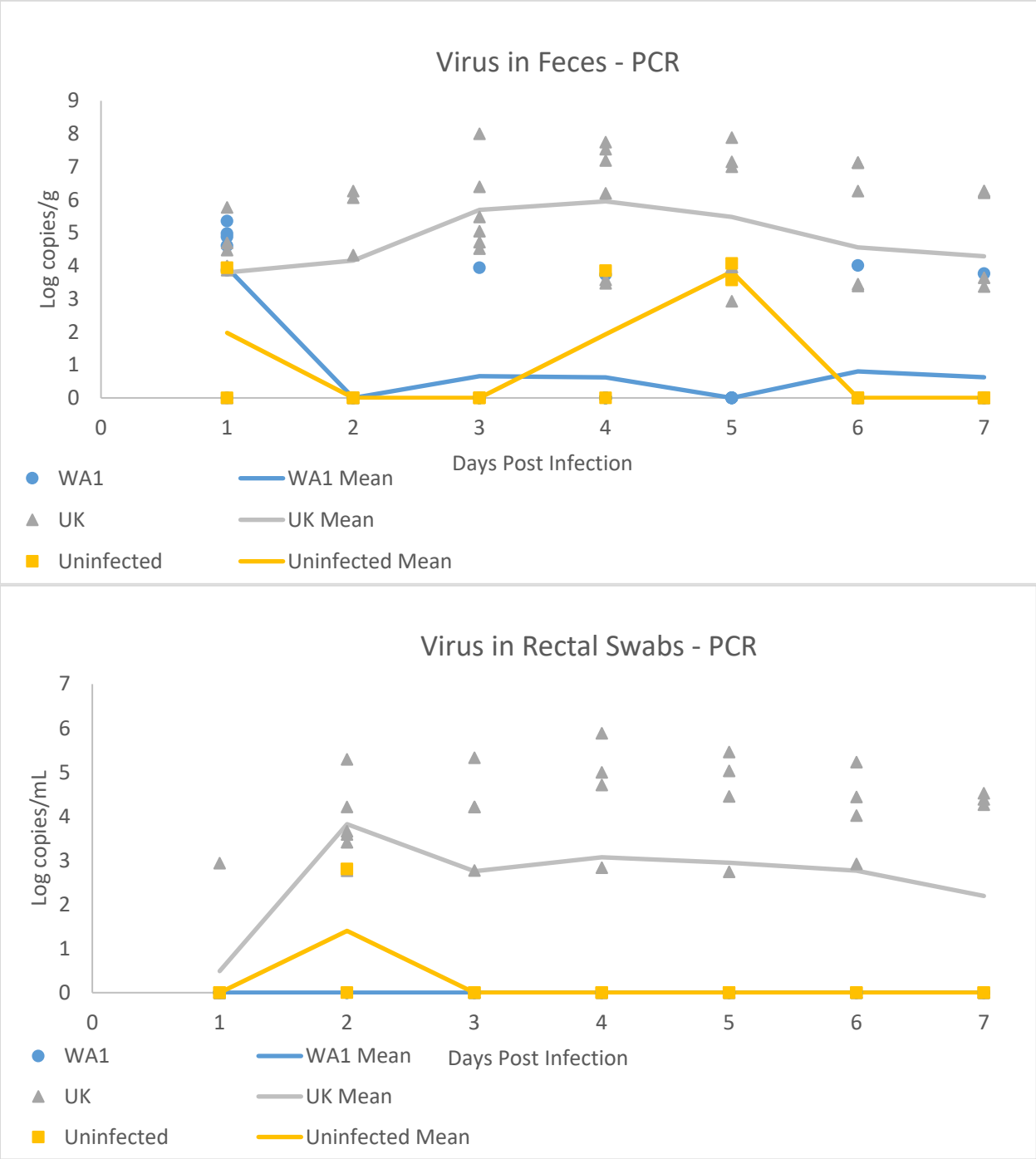

Figure 3. PCR of Feces and Rectal Swabs.
Specimens were collected daily and subjected to PCR. There teneded to be high background/low level contamination in the uninfected controls, up to 10^3 copies.

For nasal washes, the WA-1 and control hamsters showed no PCR results on Day 2 but did show low levels on Study Day 4 (10^2 to 10^3 copies/mL). This indicates a low level of cross-contamination. However, there was clear evidence of high levels (5.18×10^4 to 8.2×10^{10} copies/mL) with the UK strain, indicating that oral infection of hamsters with UK strain resulted not only in gastrointestinal infection, but also upper respiratory infection.

Table 6. Nasal Wash Titers

Animal ID	Group	Challenge Strain	Copies/mL Nasal Wash	
			Study Day 2	Study Day 4
6600	1	WA-1	-	1.20E+03
6599	1	WA-1	-	3.65E+03
6596	1	WA-1	-	6.41E+02
6589	1	WA-1	-	-
6595	1	WA-1	-	1.08E+03
6588	1	WA-1	-	1.84E+03
Group 1 (WA-1) Geomean			-	1.41E+03
6594	2	UK	1.00E+07	6.05E+10
6593	2	UK	9.31E+07	8.20E+10
6597	2	UK	2.18E+10	7.14E+10
6590	2	UK	1.03E+06	5.18E+04
6586	2	UK	8.11E+06	6.52E+04
6587	2	UK	1.63E+06	1.80E+06
Group 2 (UK) Geomean			2.55E+07	1.14E+08
6598	3	None	-	2.99E+02
6591	3	None	-	9.37E+02
Group 3 (control) Geomean			-	5.29E+02

PCR results are in Appendix F.

Section 6.
Conclusions

Oral infection of hamsters with SARS-CoV-2 UK variant resulted in infection as demonstrated by viral titers in nasal washes. There was a trend towards weight loss in the SARS-CoV-2 UK variant hamsters as well. Both body weight loss and nasal titers were only detected in males. It is unclear why the finding of infection only in maleswas observed. Virus was detected by PCR in feces (and rectal swabs and nasal washes) but not by $TCID_{50}$, suggesting that while abundant viral RNA is shed, that infectious particles may not be shed. For the UK strain, virus was also detected in nasal wash as well as $TCID_{50}$, indicating that the oral route of infection could result in respiratory infection.

The shedding persisted for 7 days in the feces and maintained a relatively constant level. This suggests a long-term infection in the gut. We suggest additional experiments to determine the duration of infection and if the gut tissues are directly infected via oral inoculation.

Oral infection of hamsters with SARS-CoV-2 WA-1 demonstrated no clinical signs and no indication of infection from PCR samples by the second day of infection. The RNA seen in feces on the first day after infection may be the original the challenge material itself with little to no replication within the hamster. Despite an ~ 10-fold higher exposure dose, our WA-1 strain produced fewer signs of infection. This is likely due to the material used on study. The particular WA-1 virus bank used on this study contained a mutation in the furan cleavage site. In that bank, 99.7% of all sequences had a mutation of the arginine 682 in the spike protein, including 35.2% which had a 36 nucleotide in-frame deletion at this position. This is considered a tissue culture adaptation, as this virus bank was produced by passaging through Vero cells. Mutations in the furan cleavage site are known to impact virulence. We selected this strain as we have previously infected hamsters with this strain via intranasal injections, but the clinical symptoms and viral titers were lower than have been reported for other SARS-CoV-2 variants. We attribute the lower amounts of viral RNA in our WA-1 infected hamster specimens to the mutation in the furan cleavage site.

Section 7.
Records

Records maintained by the testing facility included:

- Signed protocol
- Description of equipment and instruments used
- All raw data
- All correspondence, memos, notes, and telephone notes relating to the study
- Full descriptions of the experimental designs and procedures, including a description of the test equipment used, and of deviations, if any, from the protocol
- Principal mathematical equations used in generating and analyzing the data, as well as representative calculations using the equations.
- All instruments' logs and calibration information
- All SOPs applicable to this study

NOTE: All recording of data, except for automated data collection, were made directly, promptly, and legibly in ink. All data entries were dated on the date of entry and signed or initialed by the person responsible for the entry. Automated data collection systems identified the person responsible for the direct data input. Any changes to written or automated data entries were made to not obscure the original entry, indicated the reason for the change, and were signed or initialed and dated by the person responsible.

Section 8.
Quality Assurance

MRIGlobal accepts the Quality Assurance responsibility for this study. Critical phases inspected includes, but not limited to: execution of in vivo activities, necropsy activities, sample/reagent preparation and processing, sample analysis via the assays mentioned above, raw data processing and QC, and the final report.

Section 9.
Standards

This study did not require compliance with the FDA Good Laboratory Practice Regulations (21 *CFR* 58); however, the studies were performed in compliance with MRIGlobal QA procedures. All operations pertaining to this study, unless specifically defined in this protocol, were performed according to the Standard Operating Procedures of MRIGlobal.

Section 10.
Archival

All data and observations were recorded and stored at the testing facility (MRIGlobal). All data, records, documents, notes, or other materials generated by the study were collected and maintained by the study director for transfer to the MRIGlobal Records Center for archival upon completion of the study.

Appendix A.
Study Protocol

MRIGlobal Study Protocol

Title	SARS-CoV-2 Infection Via the Oral Exposure In Hamsters
MRIGlobal Project No.	110890.01.208.06.01.03
Study Director	Carl Gelhaus, Ph.D.
Testing Facility	MRIGlobal 425 Dr. Martin Luther King Jr. Blvd. Kansas City, Missouri 64110
Sponsor	US Food and Drug Administration
Proposed In Life Start Date	August 9, 2021
Proposed In Life End Date	August 17, 2021

Approvals:

Terry O'Neill for:

Claire Croutch, Ph.D.
Medical Research Portfolio Director
MRIGlobal

09 August 2021
Date

Robert Fisher -S Digitally signed by Robert Fisher -S
Date: 2021.08.09 12:11:26 -04'00'

Robert W. Fisher, Ph.D.
Senior Advisor for CBRN and Pandemic Influenza
Office of Counterterrorism and Emerging Threats (OCET)
U.S. Food and Drug Administration

09 August 2021
Date

Carl Gelhaus, Ph.D.
Study Director
MRIGlobal

8/9/21
Date

Study Protocol – MRIGlobal Project No. 110890.01.208.06.01.03

1. Objective and Justification

COVID-19 is a global public health emergency caused by the SARS-CoV-2 virus. Food safety is a major consideration during a pandemic. Food products, such as produce, may become inadvertently contaminated when displayed in grocery stores here in the United States. Moreover, a fecal-oral transmission risk may exist due to the prolonged excretion of SARS-CoV-2 from the gastrointestinal tract: several reports have identified the presence of high levels of SARS-CoV-2 RNA in feces, although infectious virus has not yet been recovered. We do not currently know the risk posed by oral exposure to the SARS-CoV-2 virus. The SARS-CoV-2 virus infects human cells by binding to the ACE2 receptor. The hamster has a similar ACE2 gene to the human and therefore is a good candidate for an animal model of COVID-19. Recent publications and our own experiments have demonstrated that hamsters develop a disease state that mimics that seen in humans infected with SARS-CoV-2 when instilled via the intranasal route. The objective of this study is examine the disease state that develops in the hamster following oral infection with SARS-CoV-2. Two variants of the SARS-CoV-2 will be tested.

2. Challenge Agents

Two isolates of the SARS-CoV-2 will be used. The first isolate challenge material will be SARS-CoV-2 isolate USA-WA1/2020 from BEI Resources. This virus has been passed through Vero cells once to obtain a master virus bank and the master virus bank was passed through Vero cells to obtain a working virus bank. The working virus bank is tested here.

Table 1. Challenge Article – WA1

Name:	SARS-CoV-2
Virus Classification:	*Coronaviridae*
Strain/Isolate:	USA-WA1/2020
Lot Number	20210214HCG
$TCID_{50}$/mL	1.67×10^{6}
Copy number/mL	1.1×10^{10}
Supplier	MRIGlobal (derived from BEI Resources)
Storage Conditions:	≤-70°C

The second isolate challenge material will be SARS-CoV-2 isolate USA/CA_CDC_5574/2020 from BEI Resources. The PANGO lineage for this isolate is the same as the UK variant (B.1.1.7). This virus has been passed through TMPRSS2 cells once to obtain a single virus bank, which is tested here.

Table 2. Challenge Article - UK

Name:	SARS-CoV-2
Virus Classification:	*Coronaviridae*
Strain/Isolate:	USA/CA_CDC_5574/2020
Lot Number	To be included in the final report
$TCID_{50}$/mL	1.1×10^7
Copy number/mL	9.0×10^{10}
Supplier	MRIGlobal (derived from BEI Resources)
Storage Conditions:	≤-70°C

3. Test System

3.1 Specifications

Species:	*Mesocricetus auratus*
Strain:	Outbred
Source:	Envigo
Number of Animals Required for Study:	7 male and 7 female
Age Range at Challenge:	6-27 weeks

Fourteen (14) Syrian hamsters will be procured, 7 male and 7 female. All animals will be placed on study.

Weight is not a criteria for this study. Any extra hamsters will be transferred off study or euthanized.

3.2 Criteria

Hamsters must be free from obvious clinical signs of ill health or malformation or any other condition that would, in the judgment of a veterinarian or the Study Director, interfere with the conduct of the study. Animals can be replaced prior to challenge, if necessary.

3.3 Animal Identification

The identity of each animal will be confirmed at each procedure by ear tag and verified against cage cards illustrating at least the animal identification number, study number, and sex.

3.4 Animal Quarantine and Acclimation

Upon delivery, animals will be inspected for signs of ill-health and quarantined/acclimated in the Spencer conventional animal facility or ABSL-3 for no less than 44-48 h. The MRIGlobal Veterinarian will examine the health of the animals and release them for the study (see SOP MRI-1501, "Acclimation and / or Quarantine Procedures for Animals").

4. Animal Housing and Environmental Conditions

4.1 Feed

All hamsters will be provided with Envigo Teklad Food or equivalent. No contaminants which could affect the results of the study will be present in the feed, as verified by the certificate of

analysis (for each lot, provided by the vendor; see SOP MRI-1510, "Procedure for Feeding Animals"). Analytical results will be retained in the facility records.

Rodent food will be provided *ad libitum* and attending veterinarian approved supplement(s) will be provided as needed for enrichment and acclimation procedures.

4.2 Water
Municipal water will be available *ad libitum*.

4.3 Housing
Hamsters have been shown to transmit SARS-CoV-2 animal to animal. Therefore, all animals will be individually housed in polycarbonate Tecniplast caging (Tecniplast, Phoenixville, PA) throughout the acclimation/quarantine period and transferred to metabolic caging prior to infection. The metabolic caging will allow for collection of feces on a daily basis. The hamsters will be housed in environmentally controlled rooms. The rooms will be maintained at a temperature of 68.0°F to 79.0°F and a relative humidity of 50% ± 20% with a 12-h light/dark cycle per day. Room temperature, humidity, and light cycles will be monitored by the OceaView® monitoring system 24-h/day (see SOP MRI-1170, "Temperature and Humidity Monitoring in Animal Rooms"). Environmental enrichment will be provided as described in SOP MRI-1505.

4.4 Veterinary Care
Discomfort and distress should be limited to that which is unavoidable in the conduct of scientifically valuable research. Animals that develop non-study related illness or injury should be evaluated by a veterinarian for determination of treatment or disposition. In such cases, and if in the opinion of the Study Director and a veterinarian, an animal is in a moribund state due to the aforementioned circumstances, that animal should be euthanized.

5. Experimental Design
Hamsters will be weighed during the quarantine and acclimation period, prior to the initiation of this protocol.

Hamsters will be exposed to virus on Study Day 0 via oral gavage. Hamsters will be individually housed in metabolic cages. Six (3 male and 3 female) hamsters will be infected with SARS-CoV-2 WA1/2020. An additional six (3 male and 3 female) hamsters will be infected with SARS-CoV-2 UK B.1.1.7 variant. In addition, one male and one female will be orally dosed with sterile DPBS media as negative controls.

Every day post-infection, feces will be collected from metabolic cages. Hamsters will be anesthetized daily of the collection of rectal swabs and body weights. Rectal swabs will be collected using viral transport media kits according to manufacturer's instructions. Animals will be monitored for 7 days post infection. On Study Days 2 and 4, hamsters will also have the nasal passages washed with phosphate buffered saline (PBS). Feces will be weighed at collection and frozen at <-60°C until analysis. Nasal washes and rectal swabs in transport media will be stored at <-60°C. All nasal washes, feces homogenates, and rectal swab solutions will be enumerated by PCR and $TCID_{50}$ assays. Animals will be euthanized and carcasses disposed without examination.

Table 3. Study Design

Group	Size (N)	Route of Viral Administration	Isolate	Study Duration	Endpoints
1	6 (3/sex)	Oral up to 1 mL; likely 1e6 $TCID_{50}$/mL Challenge Concentration	Washington (Pass 7)	7 Day	Fecal Collection, Rectal Swabs, and BW Daily and Nasal Wash on SD 2 and 4
2	6 (3/sex)		B.117 UK (Pass 4)		
3	2 (1/sex)	Oral (control – DPBS)	Control –DPBS		

6. Methods

6.1 Anesthesia

Table 4. Inhaled Anesthetic

Drug	Dose (e.g., mg/kg or mL/kg)	Dose Volume (mL)	Route	Frequency
Isoflurane	1-5%	NA	Inhaled	Daily for 8 days (Study Day 0 through 7)

Use of sedation and/or anesthesia for performing various procedures in hamsters, includes but is not limited to oral gavage challenge, nasal washes, rectal swabs, body weight measurements and prior to euthanasia.

6.2 Randomization

Animals will be weighed and allocated to challenge groups according to the study design in Table 3. Animals will be allocated to challenge groups using a randomization schedule stratified by sex and body weight, such that average body weights within each treatment group and sex will be approximately equivalent.

6.3 Oral Gavage

On the day of challenge (Study Day 0), undiluted virus will be thawed and maintained on wet ice for challenge. The UK variant virus will be diluted in DPBS to ~1 x 10^6 $TCID_{50}$/mL. The other challenge material will be diluted in DPBS to ~1x 10^5 $TCID_{50}$/mL. The dilution and confirmation of dose will be demonstrated by the $TCID_{50}$ assay of the challenge stock (i.e., back-titer) using a study specific laboratory procedure The $TCID_{50}$ of the challenge materials will be determined in a manner to allow direct comparison of the titers.

Oral gavage challenge will be conducted according to SOP MRI-1835 ("Intravenous, Subcutaneous, Intramuscular, Intraperitoneal, Dermal, Intravenous, Intratracheal, and Oral Dose Administration to Rodents"), while the animals are anesthetized with isoflurane. The challenge dose will be inoculated by oral gavage, approximately 0.8 mL using a syringe with a gavage needle.

6.4 Clinical Observations

General health observations will be performed once daily during the quarantine and acclimation period and prior to the study start. Hamsters will be observed at least twice daily after challenge from Study Day 0 through Study Day 7.

All observations will be conducted in accordance with SOP MRI-1528 "Procedure for Observation of Animals". Observations will be made without opening cages, to prevent transmission of SARS-CoV-2 infection from one animal to another.

6.5 Feces Collection

Hamsters will be individually housed in metabolic cages with a feces collection tube. Once per day, the feces will be collected into an individually labelled, pre-weighed tube for each hamster on each day. One aliquot will be made for $TCID_{50}$, a second aliquot for PCR, and all excess feces will be collected into a third tube (the excess feces tube will not be weighed). The PCR and $TCID_{50}$ tubes will be weighed after the feces is added to determine the mass of feces in each tube. Feces in tubes will be stored at ≤-60°C until analyzed.

6.6 Body Weights

Prior to all studies, hamsters will be weighed during quarantine. Body weights will be measured daily beginning on Study Day 0 through Study Day 7. Body weights will be collected in accordance with SOP MRI-2020 "Weighing Rodents and Non-rodents".

6.7 Rectal Swabs

Beginning on Study Day 1 through Study Day 7, hamsters will be anesthetized and one swab from the viral transport kit will be inserted into the rectum of one hamster and rotated briefly. The swab will be placed in the viral transport media and stored at 2-8°C until they are analyzed by $TCID_{50}$ and PCR.

6.8 Nasal Washes

Viral infection will be assessed by obtaining nasal washes on Study Days 2 and 4. Sterile DPBS will be administered intranasally to isoflurane anesthetized hamsters. Volumes for nasal wash administration will be approximately 600 µL, administered 300 µL/nostril via a 1 mL syringe attached to a 22 gauge intravenous catheter tip. The expelled fluid will be collected in a sterile petri dish and aliquoted into two cryogenic storage tubes at approximately 200 µL each. Both aliquots will be frozen at <-60°C until they are analyzed by $TCID_{50}$ and PCR (one aliquot for each assay). The nasal wash will follow the draft SOP entitled "Nasal Wash in Small Animal Models".

6.9 $TCID_{50}$

One aliquot of each feces sample, each nasal wash, and rectal swab will be used to assess presence of infectious virus, in accordance with SOP MRI-8701 "50% Tissue Culture Infectious Dose ($TCID_{50}$) Titration of Biological Safety Level (BSL)-3 Coronaviruses".

6.10 PCR

One aliquot of each feces sample, each nasal wash, and rectal swab will be used to assess presence of infectious virus in a real time quantitative PCR (RT-qPCR) assay. RT-qPCR assays

will be performed in accordance with SOP MRI-9770 "Nucleic Acid Quantification using Real-Time Polymerase Chain Reaction (PCR)".

6.11 Euthanasia

6.11.1 Unscheduled Euthanasia

The determination to euthanize an animal prior to scheduled termination will be made independently for each animal contingent on progression of disease. Animals meeting any one of the following criteria will be euthanized:

- Presence of any convulsions (denoting meningitis or encephalitis)
- Limb paralysis/paresis which prevents the animal from obtaining food and water
- Respiratory distress
- Unresponsive to touch or external stimuli
- Moribundity
- Weight loss > 20% from the baseline

All hamsters judged to meet the euthanasia criteria will be euthanized per SOP MRI-2330 "Euthanasia of Rodents". No necropsy will be performed.

6.12 Scheduled/Terminal Euthanasia and Tissue Collection

Hamsters will be euthanized on Study Day 7. Euthanasia will be performed via Isoflurane anesthesia followed by bilateral pneumothorax in accordance with SOP MRI-2330 "Euthanasia of Rodents". No necropsy will be performed.

7. Statistical Analysis of Data

7.1 Body Weight

Changes in body weight will be visualized by graphing subject-specific trajectories over time, with study groups identified using different color lines. Descriptive statistics (mean ± SD, median, minimum, and maximum) for body weight will be generated. Body weight on Study Day 0 will be considered the baseline weight. The study is not designed for statistical model analysis of body weights.

7.2 Fecal, Nasal Wash, and Rectal Swab Viral Burden

Descriptive statistics (mean ± SD, median, minimum, and maximum) for infectious viral load in feces, nasal washes, and rectal swabs will be generated for both $TCID_{50}$ and PCR results for each study group. Graphs will be generated that show the mean ± standard error of these variables as a function of sacrifice day, treatment (treated vs. placebo). Results will be visually inspected by the Study Director to assess the likelihood of statistically significant differences. Statistical analysis will be at the Study Director's discretion in consultation with the sponsor.

7.3 Clinical Observations

The number and proportion of animals showing clinical signs and symptoms of infection (*e.g.*, increased respiration, lethargy) in each study group on each study day will be tabulated.

8. Other Materials and Equipment

Materials and equipment to be used in performing the tests include but are not limited to:

- Hamsters
- Metabolic cages
- Balance
- Pipettes and tips
- Plastic vials
- Ice
- Absorbent padding
- Syringes
- Catheters for nasal washes
- Petri dishes or weigh boats to catch nasal washes
- Oral gavage needles
- Isoflurane
- PBS
- Surgical instruments
- Biosafety cabinets
- CO_2 incubators

9. SOPs

Methods and laboratory procedures to be used in performing the tests include but are not limited to the SOPs contained within animal user job roles. These include but are not limited to:

- MRI-1117, Light Cycle Maintenance in Animal rooms
- MRI-1170, Temperature and Humidity Monitoring in Animal Rooms
- MRI-1504, Procedure for Identification of Animals and Cages
- MRI-1505, Performance and Documentation of Animal Husbandry and Animal Room Maintenance
- MRI-1510, Procedure for Feeding and Watering Animals
- MRI-1528, Procedures for Observations of Animals
- MRI-1568, Procedures for Handling Rodents
- MRI-1835, Intravenous, Subcutaneous, Intramuscular, Intraperitoneal, Dermal, Intranasal, and Oral Dose Administration to Rodents
- MRI-2020, Weighing of Rodents and Non-Rodents
- MRI-2330, Euthanasia of Rodents
- MRI-8701 "50% Tissue Culture Infectious Dose (TCID50) Titration of Biological Safety Level (BSL)-3 Coronaviruses"
- MRI-9502, Data Recording Procedures for In Vitro Work Performed in Biological Safety Cabinets and In Vivo Work Performed in the Animal Facilities
- MRI-9501, Data Review Requirements for In Vitro and In Vivo Work in the Medical Countermeasures Division
- MRI-9770, Nucleic Acid Quantification using Real-Time Polymerase Chain Reaction (PCR)

10. Records to Be Maintained

Records to be maintained by the testing facility will include:

- Signed protocol and any amendments
- Description of equipment and instruments used
- All raw data
- All correspondence, memos, notes, and telephone notes relating to the study
- Full descriptions of the experimental designs and procedures, including a description of the test equipment used, and of deviations, if any, from the protocol
- All instrument logs and calibration information
- Storage conditions of test article and samples
- Test article and reagent receipt and use documentation
- Professional information about study participants
- Mathematical equations used in generating and analyzing the data as well as representative calculations using the equations
- Manufacturers information and any characterization data for the test article
- All SOPs applicable to this study

NOTE: All recording of data, except for automated data collection, will be made directly, promptly, and legibly in ink. All data entries will be dated on the date of entry and signed or initialed by the person responsible for the entry. Automated data collection systems must identify the person responsible for the direct data input. Any changes to written or automated data entries will be made so as to not obscure original entry, will indicate the reason for the change, and will be signed or initialed, and dated by the person responsible.

11. Protocol Amendments/Deviations

All changes or revisions to this protocol, and the reasons for the change, shall be documented, signed by the Study Director, dated, and maintained with the protocol, in accordance with SOP MRI-0080. If changes in the protocol are required which would change the objectives and/or scope of work, prior approval will be obtained from Sponsor.

12. Standards

This study will not require compliance with the FDA Good Laboratory Practice Regulations (21 CFR 58); however, the studies will be performed in compliance with MRIGlobal QA procedures. All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the Standard Operating Procedures of MRIGlobal, and any deviations will be documented.

13. Archival

All data and observations will be recorded and stored at the testing facility (MRIGlobal). All data, records, documents, notes, or other materials generated by the study will be collected and maintained by the study director for transfer to the MRIGlobal Records Center for archival upon completion of the study. The sponsor will receive a copy of the final report.

14. Reports

At the conclusion of each study, a brief letter report will be prepared representing the raw data and analysis.

Appendix B.
Randomization

Sex	Group	Animal ID	Body Weight (g)	Original Excel order
M	1	6600	133.2	3
M	1	6599	110.4	5
M	1	6596	98.5	7
F	1	6589	112.9	8
F	1	6595	117.8	11
F	1	6588	115.8	12
M	2	6594	108.3	4
M	2	6593	122.1	6
M	2	6597	112.7	1
F	2	6590	135.1	13
F	2	6586	108.9	14
F	2	6587	99.5	9
M	3	6598	120.5	2
F	3	6591	102.4	10

Appendix C.
Clinical Observations

SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Individual Signs - Clinical Signs

Sex: **Male** - Phase: **In-life**

Dose Group	Animal Number	Sign Type	Sign	Modifier	Remark	First Day	Last Day	Duration [Days]
G 1 / M	6600	Normal	normal			0	3	4
		Skin/Fur	Rough coat	Grade: slight		4	5	2
		Skin/Fur	Rough coat	Grade: present		5	7	3
		Found dead	scheduled sacrifice 1			7	7	1
	6599	Normal	normal			0	2	3
		Skin/Fur	Rough coat	Grade: slight		3	7	5
		Found dead	scheduled sacrifice 1			7	7	1
	6596	Normal	normal			0	1	2
		Skin/Fur	Rough coat	Grade: slight		2	5	4
		Skin/Fur	Rough coat	Grade: present		6	7	2
		Found dead	scheduled sacrifice 1			7	7	1

SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Individual Signs - Clinical Signs

Sex: **Male** - Phase: **In-life**

Dose Group	Animal Number	Sign Type	Sign	Modifier	Remark	First Day	Last Day	Duration [Days]
G 2 / M	6594	Normal	normal			0	2	3
		Skin/Fur	Rough coat	Grade: slight		3	7	5
		Found dead	scheduled sacrifice 1			7	7	1
	6593	Normal	normal			0	3	4
		Skin/Fur	Rough coat	Grade: slight		4	7	4
		Found dead	scheduled sacrifice 1			7	7	1
	6597	Normal	normal			0	1	2
		Skin/Fur	Rough coat	Grade: slight		2	2	1
		Skin/Fur	Rough coat	Grade: present		3	7	5
		Found dead	scheduled sacrifice 1			7	7	1

SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Individual Signs - Clinical Signs

Sex: **Male** - Phase: **In-life**

Dose Group	Animal Number	Sign Type	Sign	Modifier	Remark	First Day	Last Day	Duration [Days]
G 3 / M	6598	Normal	normal			0	2	3
		Skin/Fur	Rough coat	Grade: slight		2	2	1
		Skin/Fur	Rough coat	Grade: present		3	7	5
		Found dead	scheduled sacrifice 1			7	7	1

SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Individual Signs - Clinical Signs

Sex: **Female** - Phase: **In-life**

Dose Group	Animal Number	Sign Type	Sign	Modifier	Remark	First Day	Last Day	Duration [Days]
G 1 / F	6589	Normal	normal			0	1	2
		Skin/Fur	Rough coat	Grade: slight		2	7	6
		Found dead	scheduled sacrifice 1			7	7	1
	6595	Normal	normal			0	5	6
		Skin/Fur	Rough coat	Grade: slight		5	7	3
		Found dead	scheduled sacrifice 1			7	7	1
		Other	New Sign	Grade: slight	Slight Chomping	7	7	1
	6588	Normal	normal			0	7	8
		Found dead	scheduled sacrifice 1			7	7	1

SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Individual Signs - Clinical Signs

Sex: **Female** - Phase: **In-life**

Dose Group	Animal Number	Sign Type	Sign	Modifier	Remark	First Day	Last Day	Duration [Days]
G 2 / F	6590	Normal	normal			0	2	3
		Skin/Fur	Rough coat	Grade: slight		2	5	4
		Skin/Fur	Rough coat	Grade: present		6	7	2
		Found dead	scheduled sacrifice 1			7	7	1
	6586	Normal	normal			0	2	3
		Skin/Fur	Rough coat	Grade: slight		3	3	1
		Skin/Fur	Rough coat	Grade: present		4	7	4
		Found dead	scheduled sacrifice 1			7	7	1
		Eyes	squinty eye	Grade: present, Location: right		7	7	1
	6587	Normal	normal			0	6	7
		Skin/Fur	Rough coat	Grade: slight		6	7	2
		Found dead	scheduled sacrifice 1			7	7	1

SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Individual Signs - Clinical Signs

Sex: **Female** - Phase: **In-life**

Dose Group	Animal Number	Sign Type	Sign	Modifier	Remark	First Day	Last Day	Duration [Days]
G 3 / F	6591	Normal	normal			0	7	8
		Found dead	scheduled sacrifice 1			7	7	1

Appendix D.
Body Weights

FDA 1

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Ascentos™ 1.3

SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Individual Body Weights - BW / Body Weights [g]

Sex: **Male** - Phase: **In-life**

Dose Group	Animal Number	day 0 [1 Time Daily]	day 1 [1 Time Daily]	day 2 [1 Time Daily]	day 3 [1 Time Daily]	day 4 [1 Time Daily]	day 5 [1 Time Daily]	day 6 [1 Time Daily]	day 7 [1 Time Daily]
	6600	133.7	133.2	131.1	129.3	130.5	132.5	131.4	130.3
	6599	114.7	109.5	107.1	101.7	107.8	110.7	110	111.1
	6596	101.4	100.7	100.6	103.7	100.8	100.6	101	100.7
	Mean	116.6	114.47	112.93	111.57	113.03	114.6	114.13	114.03
	S.d.	16.23	16.81	16.06	15.39	15.53	16.3	15.62	15.02
G 1 / M	N	3	3	3	3	3	3	3	3

FDA 2

Study 110890.01.208.06.01.03 10/26/2021 9:16

Ascentos™ 1.3

SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Individual Body Weights - BW / Body Weights [g]

Sex: **Male** - Phase: **In-life**

Dose Group	Animal Number	day 0 [1 Time Daily]	day 1 [1 Time Daily]	day 2 [1 Time Daily]	day 3 [1 Time Daily]	day 4 [1 Time Daily]	day 5 [1 Time Daily]	day 6 [1 Time Daily]	day 7 [1 Time Daily]
	6594	115.7	111.9	107.7	112.9	113.2	111.7	110	109.9
	6593	125.2	125.3	126.8	104.3	119.6	115.3	111.2	106.3
	6597	119.1	114.6	109.5	106.9	112.9	102	99	95.7
	Mean	120	117.27	114.67	108.03	115.23	109.67	106.73	103.97
	S.d.	4.81	7.09	10.55	4.41	3.78	6.88	6.72	7.38
G 2 / M	N	3	3	3	3	3	3	3	3

FDA 3

Study 110890.01.208.06.01.03 10/26/2021 9:16

Ascentos™ 1.3

SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Individual Body Weights - BW / Body Weights [g]

Sex: **Male** - Phase: **In-life**

Dose Group	Animal Number	day 0 [1 Time Daily]	day 1 [1 Time Daily]	day 2 [1 Time Daily]	day 3 [1 Time Daily]	day 4 [1 Time Daily]	day 5 [1 Time Daily]	day 6 [1 Time Daily]	day 7 [1 Time Daily]
	6598	122.6	119	115.4	115.3	117.8	116.9	117.1	117.4
	Mean	122.6	119	115.4	115.3	117.8	116.9	117.1	117.4
	S.d.								
G 3 / M	N	1	1	1	1	1	1	1	1

FDA 4

Study 110890.01.208.06.01.03 10/26/2021 9:16

Ascentos™ 1.3

SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Individual Body Weights - BW / Body Weights [g]

Sex: **Female** - Phase: **In-life**

Dose Group	Animal Number	day 0 [1 Time Daily]	day 1 [1 Time Daily]	day 2 [1 Time Daily]	day 3 [1 Time Daily]	day 4 [1 Time Daily]	day 5 [1 Time Daily]	day 6 [1 Time Daily]	day 7 [1 Time Daily]
	6589	127.7	128.9	130	130.1	128.8	129.7	133.6	135.2
	6595	123.2	120.7	121.1	120.6	123.3	122.4	122.3	123.2
	6588	117.8	114	109.1	105.4	110.4	114.9	116.6	118.3
	Mean	122.9	121.2	120.07	118.7	120.83	122.33	124.17	125.57
	S.d.	4.96	7.46	10.49	12.46	9.44	7.4	8.65	8.7
G 1 / F	N	3	3	3	3	3	3	3	3

SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Individual Body Weights - BW / Body Weights [g]

Sex: **Female** - Phase: **In-life**

Dose Group	Animal Number	day 0 [1 Time Daily]	day 1 [1 Time Daily]	day 2 [1 Time Daily]	day 3 [1 Time Daily]	day 4 [1 Time Daily]	day 5 [1 Time Daily]	day 6 [1 Time Daily]	day 7 [1 Time Daily]
	6590	133.2	126.2	130.1	132.4	133.9	133.8	124.5	134.1
	6586	111.4	107.3	104.6	103.7	108.4	111.1	111.7	111.2
	6587	114	113.4	110.5	110.1	112.2	112.7	112.2	111.4
	Mean	119.53	115.63	115.07	115.4	118.17	119.2	116.13	118.9
	S.d.	11.91	9.65	13.35	15.07	13.76	12.67	7.25	13.16
G 2 / F	N	3	3	3	3	3	3	3	3

SARS-CoV-2 Infection Via the Oral Exposure In Hamsters

Individual Body Weights - BW / Body Weights [g]

Sex: **Female** - Phase: **In-life**

Dose Group	Animal Number	day 0 [1 Time Daily]	day 1 [1 Time Daily]	day 2 [1 Time Daily]	day 3 [1 Time Daily]	day 4 [1 Time Daily]	day 5 [1 Time Daily]	day 6 [1 Time Daily]	day 7 [1 Time Daily]
	6591	109	110.5	109.3	111.5	107.3	107.8	106.4	107.5
	Mean	109	110.5	109.3	111.5	107.3	107.8	106.4	107.5
	S.d.								
G 3 / F	N	1	1	1	1	1	1	1	1

Appendix E.
$TCID_{50}$ Results

Sample Number	Animal ID	Group	Study Day	Initial Dilution	TCID50/ml	Log10 TCID50/mL
1	6600	1	1	200/700	1.11E+01	1.04
2	6599	1	1	200/700	1.11E+01	1.04
3	6596	1	1	200/700	1.11E+01	1.04
4	6589	1	1	200/700	1.11E+01	1.04
5	6595	1	1	200/700	1.11E+01	1.04
6	6588	1	1	200/700	1.11E+01	1.04
7	6594	2	1	200/700	1.11E+01	1.04
8	6593	2	1	200/700	1.11E+01	1.04
9	6597	2	1	200/700	1.11E+01	1.04
10	6590	2	1	200/700	1.11E+01	1.04
11	6586	2	1	200/700	1.11E+01	1.04
12	6587	2	1	200/700	1.11E+01	1.04
13	6598	3	1	200/700	1.11E+01	1.04
14	6591	3	1	200/700	1.11E+01	1.04
15	6600	1	2	200/700	1.11E+01	1.04
16	6599	1	2	200/700	1.11E+01	1.04
17	6596	1	2	200/700	1.11E+01	1.04
18	6589	1	2	200/700	1.11E+01	1.04
19	6595	1	2	200/700	1.11E+01	1.04
20	6588	1	2	200/700	1.11E+01	1.04
21	6594	2	2	200/700	1.11E+01	1.04
22	6593	2	2	200/700	1.11E+01	1.04
23	6597	2	2	200/700	1.11E+01	1.04
24	6590	2	2	200/700	1.11E+01	1.04
25	6586	2	2	200/700	1.11E+01	1.04
26	6587	2	2	200/700	1.11E+01	1.04
27	6598	3	2	200/700	1.11E+01	1.04
28	6591	3	2	200/700	1.11E+01	1.04
29	6600	1	3	200/700	1.11E+01	1.04
30	6599	1	3	200/700	1.11E+01	1.04
31	6596	1	3	200/700	1.11E+01	1.04
32	6589	1	3	200/700	1.11E+01	1.04
33	6595	1	3	200/700	1.11E+01	1.04
34	6588	1	3	200/700	1.11E+01	1.04
35	6594	2	3	200/700	1.11E+01	1.04

Sample Number	Animal ID	Group	Study Day	Initial Dilution	TCID50/ml	Log10 TCID50/mL
36	6593	2	3	200/700	1.11E+01	1.04
37	6597	2	3	200/700	1.11E+01	1.04
38	6590	2	3	200/700	1.11E+01	1.04
39	6586	2	3	200/700	1.11E+01	1.04
40	6587	2	3	200/700	1.11E+01	1.04
41	6598	3	3	200/700	1.11E+01	1.04
42	6591	3	3	200/700	1.11E+01	1.04
43	6600	1	4	200/700	1.11E+01	1.04
44	6599	1	4	200/700	1.11E+01	1.04
45	6596	1	4	200/700	1.11E+01	1.04
46	6589	1	4	200/700	1.11E+01	1.04
47	6595	1	4	200/700	1.11E+01	1.04
48	6588	1	4	200/700	1.11E+01	1.04
49	6594	2	4	200/700	1.11E+01	1.04
50	6593	2	4	200/700	1.11E+01	1.04
51	6597	2	4	200/700	1.11E+01	1.04
52	6590	2	4	200/700	1.11E+01	1.04
53	6586	2	4	200/700	1.11E+01	1.04
54	6587	2	4	200/700	1.11E+01	1.04
55	6598	3	4	200/700	1.11E+01	1.04
56	6591	3	4	200/700	1.11E+01	1.04
57	6600	1	5	200/700	1.11E+01	1.04
58	6599	1	5	200/700	1.11E+01	1.04
59	6596	1	5	200/700	1.11E+01	1.04
60	6589	1	5	200/700	1.11E+01	1.04
61	6595	1	5	200/700	1.11E+01	1.04
62	6588	1	5	200/700	1.11E+01	1.04
63	6594	2	5	200/700	1.11E+01	1.04
64	6593	2	5	200/700	1.11E+01	1.04
65	6597	2	5	200/700	1.11E+01	1.04
66	6590	2	5	200/700	1.11E+01	1.04
67	6586	2	5	200/700	1.11E+01	1.04
68	6587	2	5	200/700	1.11E+01	1.04
69	6598	3	5	200/700	1.11E+01	1.04
70	6591	3	5	200/700	1.11E+01	1.04

Sample Number	Animal ID	Group	Study Day	Initial Dilution	TCID50/ml	Log10 TCID50/mL
71	6600	1	6	200/700	1.11E+01	1.04
72	6599	1	6	200/700	1.11E+01	1.04
73	6596	1	6	200/700	1.11E+01	1.04
74	6589	1	6	200/700	1.11E+01	1.04
75	6595	1	6	200/700	1.11E+01	1.04
76	6588	1	6	200/700	1.11E+01	1.04
77	6594	2	6	200/700	1.11E+01	1.04
78	6593	2	6	200/700	3.50E+01	1.54
79	6597	2	6	200/700	1.11E+01	1.04
80	6590	2	6	200/700	1.11E+01	1.04
81	6586	2	6	200/700	1.11E+01	1.04
82	6587	2	6	200/700	1.11E+01	1.04
83	6598	3	6	200/700	1.11E+01	1.04
84	6591	3	6	200/700	1.11E+01	1.04
85	6600	1	7	200/700	1.11E+01	1.04
86	6599	1	7	200/700	1.11E+01	1.04
87	6596	1	7	200/700	1.11E+01	1.04
88	6589	1	7	200/700	1.11E+01	1.04
89	6595	1	7	200/700	1.11E+01	1.04
90	6588	1	7	200/700	1.11E+01	1.04
91	6594	2	7	200/700	1.11E+01	1.04
92	6593	2	7	200/700	1.11E+01	1.04
93	6597	2	7	200/700	1.11E+01	1.04
94	6590	2	7	200/700	1.11E+01	1.04
95	6586	2	7	200/700	1.11E+01	1.04
96	6587	2	7	200/700	1.11E+01	1.04
97	6598	3	7	200/700	1.11E+01	1.04
98	6591	3	7	200/700	1.11E+01	1.04
					≤	

Sample Number	Animal ID	Group	Study Day	Dilution	Dilution for Reed Muench	TCID50/ml	Log10 TCID50/mL
1	6591	3	2	100/700	1.43E-01	2.21E+01	1.35
2	6589	3	2	100/700	1.43E-01	2.21E+01	1.35
3	6600	1	2	100/700	1.43E-01	2.21E+01	1.35
4	6599	1	2	100/700	1.43E-01	2.21E+01	1.35
5	6596	1	2	100/700	1.43E-01	2.21E+01	1.35
6	6589	1	2	100/700	1.43E-01	2.21E+01	1.35
7	6588	1	2	25/700	3.57E-02	8.85E+01	1.95
8	6595	1	2	100/700	1.43E-01	2.21E+01	1.35
9	6597 (6585)	2	2	100/700	1.43E-01	4.77E+02	2.68
10	6594	2	2	100/700	1.43E-01	2.95E+01	1.47
11	6593	2	2	100/700	1.43E-01	2.21E+01	1.35
12	6587	2	2	100/700	1.43E-01	2.21E+01	1.35
13	6590	2	2	75/700	1.07E-01	2.95E+01	1.47
14	6586	2	2	50/700	7.14E-02	4.43E+01	1.65
15	6591	3	4	100/700	1.43E-01	2.21E+01	1.35
16	6598	3	4	100/700	1.43E-01	2.21E+01	1.35
17	6600	1	4	100/700	1.43E-01	2.21E+01	1.35
18	6599	1	4	100/700	1.43E-01	2.21E+01	1.35
19	6596	1	4	100/700	1.43E-01	2.21E+01	1.35
20	6589	1	4	100/700	1.43E-01	2.21E+01	1.35
21	6588	1	4	100/700	1.43E-01	2.21E+01	1.35
22	6595	1	4	100/700	1.43E-01	2.21E+01	1.35
23	6597 (6585)	2	4	100/700	1.43E-01	2.21E+02	2.35
24	6594	2	4	100/700	1.43E-01	2.21E+03	3.35
25	6593	2	4	100/700	1.43E-01	2.21E+02	2.35
26	6587	2	4	100/700	1.43E-01	2.21E+01	1.35
27	6590	2	4	100/700	1.43E-01	2.21E+01	1.35
28	6586	2	4	100/700	1.43E-01	2.21E+01	1.35
						≤	

Sample Number	Animal ID	Group	Study Day	Initial Dilution	TCID50/ml	Log10 TCID50/mL
1	6600	1	1	Neat	3.16E+00	0.50
2	6599	1	1	Neat	3.16E+00	0.50
3	6596	1	1	Neat	3.16E+00	0.50
4	6589	1	1	Neat	3.16E+00	0.50
5	6595	1	1	Neat	3.16E+00	0.50
6	6588	1	1	Neat	3.16E+00	0.50
7	6594	2	1	Neat	3.16E+00	0.50
8	6593	2	1	Neat	3.16E+00	0.50
9	6597	2	1	Neat	3.16E+00	0.50
10	6590	2	1	Neat	3.16E+00	0.50
11	6586	2	1	Neat	3.16E+00	0.50
12	6587	2	1	Neat	3.16E+00	0.50
13	6598	3	1	Neat	3.16E+00	0.50
14	6591	3	1	Neat	3.16E+00	0.50
15	6600	1	2	Neat	3.16E+00	0.50
16	6599	1	2	Neat	3.16E+00	0.50
17	6596	1	2	Neat	3.16E+00	0.50
18	6589	1	2	Neat	3.16E+00	0.50
19	6595	1	2	Neat	3.16E+00	0.50
20	6588	1	2	Neat	3.16E+00	0.50
21	6594	2	2	Neat	3.16E+00	0.50
22	6593	2	2	Neat	3.16E+00	0.50
23	6597	2	2	Neat	3.16E+00	0.50
24	6590	2	2	Neat	3.16E+00	0.50
25	6586	2	2	Neat	3.16E+00	0.50
26	6587	2	2	Neat	3.16E+00	0.50
27	6598	3	2	Neat	3.16E+00	0.50
28	6591	3	2	Neat	3.16E+00	0.50
29	6600	1	3	Neat	3.16E+00	0.50
30	6599	1	3	Neat	3.16E+00	0.50
31	6596	1	3	Neat	3.16E+00	0.50
32	6589	1	3	Neat	3.16E+00	0.50
33	6595	1	3	Neat	3.16E+00	0.50
34	6588	1	3	Neat	3.16E+00	0.50

Sample Number	Animal ID	Group	Study Day	Initial Dilution	TCID50/ml	Log10 TCID50/mL
35	6594*	2	3	Neat	3.16E+00	0.50
36	6594*	2	3	Neat	3.16E+00	0.50
37	6593*	2	3	Neat	3.16E+00	0.50
38	6593*	2	3	Neat	3.16E+00	0.50
40	6597	2	3	Neat	3.16E+00	0.50
41	6590	2	3	Neat	3.16E+00	0.50
42	6598	3	3	Neat	3.16E+00	0.50
43	6591	3	3	Neat	3.16E+00	0.50
44	6600	1	4	Neat	3.16E+00	0.50
45	6599	1	4	Neat	3.16E+00	0.50
46	6596	1	4	Neat	3.16E+00	0.50
47	6589	1	4	Neat	3.16E+00	0.50
48	6595	1	4	Neat	3.16E+00	0.50
49	6588	1	4	Neat	3.16E+00	0.50
50	6594	2	4	Neat	3.16E+00	0.50
51	6593	2	4	Neat	3.16E+00	0.50
52	6597	2	4	Neat	3.16E+00	0.50
53	6590	2	4	Neat	3.16E+00	0.50
54	6586	2	4	Neat	3.16E+00	0.50
55	6587	2	4	Neat	3.16E+00	0.50
56	6598	3	4	Neat	3.16E+00	0.50
57	6591	3	4	Neat	3.16E+00	0.50
58	6600	1	5	Neat	3.16E+00	0.50
59	6599	1	5	Neat	3.16E+00	0.50
60	6596	1	5	Neat	3.16E+00	0.50
61	6589	1	5	Neat	3.16E+00	0.50
62	6595	1	5	Neat	3.16E+00	0.50
63	6588	1	5	Neat	3.16E+00	0.50
64	6594	2	5	Neat	3.16E+00	0.50
65	6593	2	5	Neat	3.16E+00	0.50
66	6597	2	5	Neat	3.16E+00	0.50
67	6590	2	5	Neat	3.16E+00	0.50
68	6587	2	5	Neat	3.16E+00	0.50

* - There were two tubes labelled 6594 and two tubes labelled 6593. There were no tues labelled 6586 or 6587. All IDs were from Group 2. Presumably the tubes for 6586 and 6587 were mislabelled. There is no impact, as all samples were negative.

Sample Number	Animal ID	Group	Study Day	Initial Dilution	TCID50/ml	Log10 TCID50/mL
69	6586	2	5	Neat	3.16E+00	0.50
70	6598	3	5	Neat	3.16E+00	0.50
71	6591	3	5	Neat	3.16E+00	0.50
72	6600	1	6	Neat	3.16E+00	0.50
73	6599	1	6	Neat	3.16E+00	0.50
74	6596	1	6	Neat	3.16E+00	0.50
75	6589	1	6	Neat	3.16E+00	0.50
76	6595	1	6	Neat	3.16E+00	0.50
77	6588	1	6	Neat	3.16E+00	0.50
78	6594	2	6	Neat	3.16E+00	0.50
79	6593	2	6	Neat	3.16E+00	0.50
80	6597	2	6	Neat	3.16E+00	0.50
81	6590	2	6	Neat	3.16E+00	0.50
82	6586	2	6	Neat	3.16E+00	0.50
83	6587	2	6	Neat	3.16E+00	0.50
84	6598	3	6	Neat	3.16E+00	0.50
85	6591	3	6	Neat	3.16E+00	0.50
86	6600	1	7	Neat	3.16E+00	0.50
87	6599	1	7	Neat	3.16E+00	0.50
88	6596	1	7	Neat	3.16E+00	0.50
89	6589	1	7	Neat	3.16E+00	0.50
90	6595	1	7	Neat	3.16E+00	0.50
91	6588	1	7	Neat	3.16E+00	0.50
92	6594	2	7	Neat	3.16E+00	0.50
93	6593	2	7	Neat	3.16E+00	0.50
94	6597	2	7	Neat	3.16E+00	0.50
95	6590	2	7	Neat	3.16E+00	0.50
96	6586	2	7	Neat	3.16E+00	0.50
97	6587	2	7	Neat	3.16E+00	0.50
98	6598	3	7	Neat	3.16E+00	0.50
98	6591	3	7	Neat	3.16E+00	0.50
					≤	

Appendix F.
PCR Results

Fecal Swab Sample Results (cpy/mL)

Animal ID	Group	Challenge Strain	Study Day						
			SD1	SD2	SD3	SD4	SD5	SD6	SD7
6598	3	None	-	-	6.37E+02	-	-	-	-
6591	3	None	-	-	-	-	-	-	-
6600	1	WA-1	-	-	-	-	-	-	-
6599	1	WA-1	-	-	-	-	-	-	-
6596	1	WA-1	-	-	-	-	-	-	-
6589	1	WA-1	-	-	-	-	-	-	-
6595	1	WA-1	-	-	-	-	-	-	-
6588	1	WA-1	-	-	-	-	-	-	-
6594 (SD3: 6594)	2	UK	-	1.63E+04	5.91E+02	5.14E+04	2.87E+05	1.69E+05	2.42E+04
6593 (SD3: 6594D)	2	UK	-	3.89E+03	-	9.96E+04	2.83E+04	2.74E+04	1.84E+04
6597 (SD3: 6593)	2	UK	8.64E+02	1.95E+05	1.63E+04	7.63E+05	1.07E+05	1.04E+04	3.34E+04
6590 (SD3: 6593D)	2	UK	-	4.59E+03	2.13E+05	-	5.53E+02	8.29E+02	-
6586 (SD3: 6597)	2	UK	-	2.58E+03	1.09E+06	-	-	-	-
6587 (SD3: 6590)	2	UK	-	5.85E+02		6.82E+02		-	-

(1) Animal ID's with SD3 note were mislabeled and true identity cannot be verified.
The order of the tubes was used to assume actual Animal ID's but what was written on the tube is shown in the SD3 note. "D" signifies duplicate labels.

Stool Sample Results (cpy/g)

Animal ID	Group	Challenge Strain	Study Day						
			SD1	SD2	SD3	SD4	SD5	SD6	SD7
6598	3	None	-	-	-	-	1.18E+04	-	-
6591	3	None	8.71E+03	-	-	7.14E+03	3.72E+03	-	-
6600	1	WA-1	9.49E+04	-	-	-	-	-	-
6599	1	WA-1	7.56E+04	-	-	-	-		5.80E+03
6596	1	WA-1	2.26E+05	-	8.75E+03	5.41E+03	-	-	-
6589	1	WA-1	7.32E+03	-	-	-	-	1.03E+04	-
6595	1	WA-1		-	-	-	-	-	-
6588	1	WA-1		-	-	-	-	-	-
6594	2	UK	4.95E+04	-	5.26E+04	5.61E+07	7.69E+07	1.33E+07	1.86E+06
6593	2	UK	3.02E+04	1.16E+06	2.49E+06	1.56E+07	1.00E+07	1.85E+06	1.63E+06
6597	2	UK	5.87E+05	1.83E+06	1.00E+08	3.43E+07	1.42E+07	1.36E+07	1.75E+06
6590	2	UK	7.38E+03	2.11E+04	1.12E+05	2.96E+03	9.37E+03	-	-
6586	2	UK	-		3.03E+05	1.58E+06	9.19E+03	2.80E+03	4.36E+03
6587	2	UK	9.85E+03		3.34E+04	3.76E+03	8.46E+02	2.45E+03	2.38E+03

Animal ID	Group	Challenge Strain	Copies/mL Nasal Wash	
			Study Day 2	Study Day 4
6600	1	WA-1	-	1.20E+03
6599	1	WA-1	-	3.65E+03
6596	1	WA-1	-	6.41E+02
6589	1	WA-1	-	-
6595	1	WA-1	-	1.08E+03
6588	1	WA-1	-	1.84E+03
Group 1 (WA-1) Geomean			-	1.41E+03
6594	2	UK	1.00E+07	6.05E+10
6593	2	UK	9.31E+07	8.20E+10
6597	2	UK	2.18E+10	7.14E+10
6590	2	UK	1.03E+06	5.18E+04
6586	2	UK	8.11E+06	6.52E+04
6587	2	UK	1.63E+06	1.80E+06
Group 2 (UK) Geomean			2.55E+07	1.14E+08
6598	3	None	-	2.99E+02
6591	3	None	-	9.37E+02
Group 3 (control) Geomean			-	5.29E+02