Materials and Methods

Biosafety and ethics. All SARS-CoV-2 studies were approved by the Institutional Biosafety Committee (IBC) and performed in high biocontainment (BSL3/BSL4) at Rocky Mountain Laboratories (RML), NIAID, NIH. All sample processing in high biocontainment and sample removal followed IBC-approved Standard Operating Protocols (SOPs) (*1*). All experiments involving AGMs were performed in strict accordance with approved Institutional Animal Care and Use Committee protocols and following recommendations from the Guide for the Care and Use of Laboratory Animals of the Office of Animal Welfare, National Institutes of Health and the Animal Welfare Act of the US Department of Agriculture, in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-accredited facility. AGMs were placed in a climate-controlled room with a fixed 12-hour light-dark cycle. Animals were singly housed in adjacent primate cages allowing social interactions and provided with commercial monkey chow, treats, and fruit twice daily with water *ad libitum*. Environmental enrichment was provided with a variety of human interaction, manipulanda, commercial toys, movies, and music. AGMs were monitored at least twice daily throughout the study.

Virus and cells. SARS-CoV-2 isolate SARS-CoV-2/human/USA/RML-7/2020 (MW127503.1), strain D614G, was obtained from a nasopharyngeal swab obtained on July 19, 2020. Sequencing of the viral stock showed it to be 100% identical to the deposited Genbank sequence and no contaminants were detected (*2*). SARS-CoV-2 variant B.1.1.7 (hCoV-19/England/204820464/2020, EPI_ISL_683466) was obtained from Public Health England via BEI Resources (Manassas, VA, USA). The supplied passage 2 material was propagated once in

Vero E6 cells. Sequencing confirmed the presence of three SNPs in this stock: nsp6 D156G (present in 14% of all reads), nsp6 L257F (18%) and nsp7 V11I (13%) (*3*).

Virus propagation was performed in Vero E6 cells in DMEM (Sigma-Aldrich, St Louis, MO, USA) supplemented with 2% fetal bovine serum, 1 mM L-glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin (DMEM2). Vero E6 cells were maintained in DMEM supplemented with 10% fetal bovine serum, 1 mM L-glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin (DMEM10). Mycoplasma testing of cell lines and viral stocks is performed regularly, and no mycoplasma was detected.

Study design. Eleven SARS-CoV-2 seronegative AGMs (3.8-6.7 kg) were divided into 2 groups for infection with either the contemporary D614G strain (RML7) (n=5) or the recently emerged B.1.1.7 (UK variant) (n=6). A Nasal Mucosal Atomization Device (Teleflex, MAD110) was used to deliver 10^6 infectious particles ($5x10^5$ per naris diluted in 500ul DMEM with no additives). Clinical examinations were performed on days 0, 1, 3, 5 and 7. Blood and serum were collected for hematology, blood chemistry, coagulation and virological analysis. Oral, nasal and rectal swabs were collected at every examination for virological analysis. Bronchial cytology brushes were collected on days 3, 5 and 7 and bronchioalveolar lavage (BAL) samples were also collected on days 3 and 5 for virological analysis. Tissues were collected following euthanasia on day 7 for pathology and virological analysis. Studies were performed in successive weeks and different animal study groups to avoid contamination between studies, the D416G study was run first followed by the B.1.1.7 study.

Virus titration. Virus isolation was performed on tissues following homogenization in 1 mL DMEM using a TissueLyser (Qiagen, Germantown, MD, USA) and inoculating Vero E6 cells in

a 96 well plate with 200 µL of 1:10 serial dilutions of the homogenate. One hour following inoculation of cells, the inoculum was removed and replaced with 200 µL DMEM. Virus isolation of blood and swab samples were performed in a similar manner. Samples were vortexed for 30 seconds before performing the 1:10 dilution series. The inoculum (200ul) was placed on cells and rocked for 1h. Infectious supernatant was removed and replaced with fresh DMEM. Seven days following inoculation, cytopathogenic effect was scored and the $TCID_{50}$ was calculated using the Reed-Muench formula (*4*).

Viral RNA detection. qPCR was performed on RNA samples extracted from swabs or tissues using QiaAmp Viral RNA or RNeasy kits, respectively (Qiagen, Germantown, MD, USA). Viral RNA was detected with one-step real-time RT-PCR assays designed to amplify total viral RNA (N gene) (*5*) or sgRNA by amplifying a region of E gene to detect replicating virus (*6*). Dilutions of RNA standards counted by droplet digital PCR were run in parallel and used to calculate viral RNA genome copies. A Rotor-Gene probe kit (Qiagen, Germantown, MD, USA) was used to run the PCRs according to the instructions of the manufacturer.

Hematology, Serum Chemistry and Coagulation. Hematology analysis was completed on a ProCyte DX (IDEXX Laboratories, Westbrook, ME, USA) and the following parameters were evaluated: red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution weight (RDW), platelets, mean platelet volume (MPV), white blood cells (WBC), neutrophil count (abs and %), lymphocyte count (abs and %), monocyte count (abs and %), eosinophil count (abs and %), and basophil count (abs and %). Serum chemistries were completed on a VetScan VS2 Chemistry Analyzer (Abaxis, Union City, CA, USA) and the following parameters were evaluated: glucose, blood urea nitrogen (BUN),

creatinine, calcium, albumin, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, globulin, sodium, potassium, chloride, and total carbon dioxide. Coagulation values were determined from citrated plasma utilizing a STart4 Hemostatis Analyzer and associated testing kits (Diagnostica Stago, Parsippany, NJ, USA).

Cytokine analyses. Concentrations of cytokines and chemokines present in the serum from SARS-CoV-2 infected AGMs were quantified using a multiplex bead-based assay (1:4 dilution)- the LEGENDPlex Non-Human Primate Cytokine/Chemokines 13-plex (BioLegend, San Diego, CA USA). Analytes detected by this panel are the following: IFN-γ, IL-1β, IL-6, IL-8, MCP-1, MIP-1α, MIP-1β, MIG, TNF-α, I-TAC, RANTES, IP-10, and Eotaxin. Samples were diluted 1:4 in duplicate prior to processing according the manufacturer's instructions. Samples were read using the BD FACS Symphony instrument (BD Biosciences, San Jose, CA USA) and analyzed using LEGENDplexTM Data Analysis Software following data acquisition.

Thoracic radiographs. Ventro-dorsal and right/left lateral radiographs were taken on clinical exam days prior to any other procedures (e.g. bronchoalveolar lavage, nasal flush). Radiographs were evaluated and scored for the presence of pulmonary infiltrates by two board-certified clinical veterinarians according to a previously published standard scoring system (*7*). Briefly, each lung lobe (upper left, middle left, lower left, upper right, middle right, lower right) was scored individually based on the following criteria: 0 = normal examination; 1 = mild interstitial pulmonary infiltrates; 2 = moderate interstitial pulmonary infiltrates, perhaps with partial cardiac border effacement and small areas of pulmonary consolidation (alveolar patterns and air bronchograms); and 3 = pulmonary consolidation as the primary lung pathology, seen as a progression from grade 2 lung pathology. At study completion, thoracic radiograph findings

were reported as a single radiograph score for each animal on each exam day. To obtain this score, the scores assigned to each of the six lung lobes were added together and recorded as the radiograph score for each animal on each exam day. Scores range from 0 to 18 for each animal on each exam day.

Histology and Immunohistochemistry. Tissues were fixed in 10 % neutral buffered formalin with two changes, for a minimum of 7 days according to an IBC-approved SOP. Tissues were processed with a Sakura VIP-6 Tissue Tek, on a 12-hour automated schedule, using a graded series of ethanol, xylene, and PureAffin. Embedded tissues were sectioned at 5 µm and dried overnight at 42°C prior to staining with hematoxylin and eosin. Specific staining was detected using SARS-CoV/SARS-CoV-2 nucleocapsid antibody (Sino Biological cat#40143-MM05) at a 1:1000 dilution. The tissues were processed for immunohistochemistry using the Discovery Ultra automated stainer (Ventana Medical Systems) with a ChromoMap DAB kit (Roche Tissue Diagnostics cat#760–159) (Roche Diagnostics Corp., Indianapolis, IN, USA).

Statistical analyses. Statistical analysis was performed in Prism 8 (GraphPad, San Diego, CA, USA). Multiple t-tests were used to assess statistical significance between the two infection groups.

Supplementary Figures

Fig S1

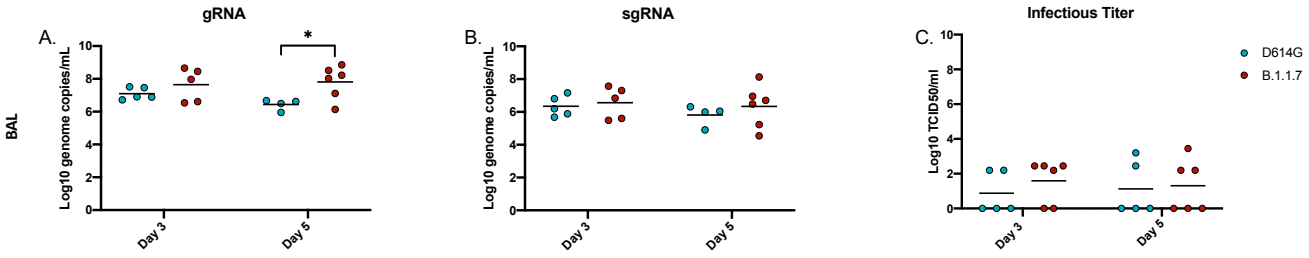

Figure S1: Viral loads in the lower respiratory system (BAL).

AGMs were infected with either the D614G or B.1.1.7 SARS-CoV-2 variant intranasally utilizing a Nasal Mucosal Atomization Device. Bronchioalveolar lavage (BAL) samples were collected on days 3 and 5 post-infection and measured for gRNA, sgRNA and infectious titers. (A-C) A significant difference in gRNA collected in BAL samples was detected on day 5 post-infection (*p-value <0.05), no other significant differences were detected. Multiple t-tests were used to compare groups.

Fig S2

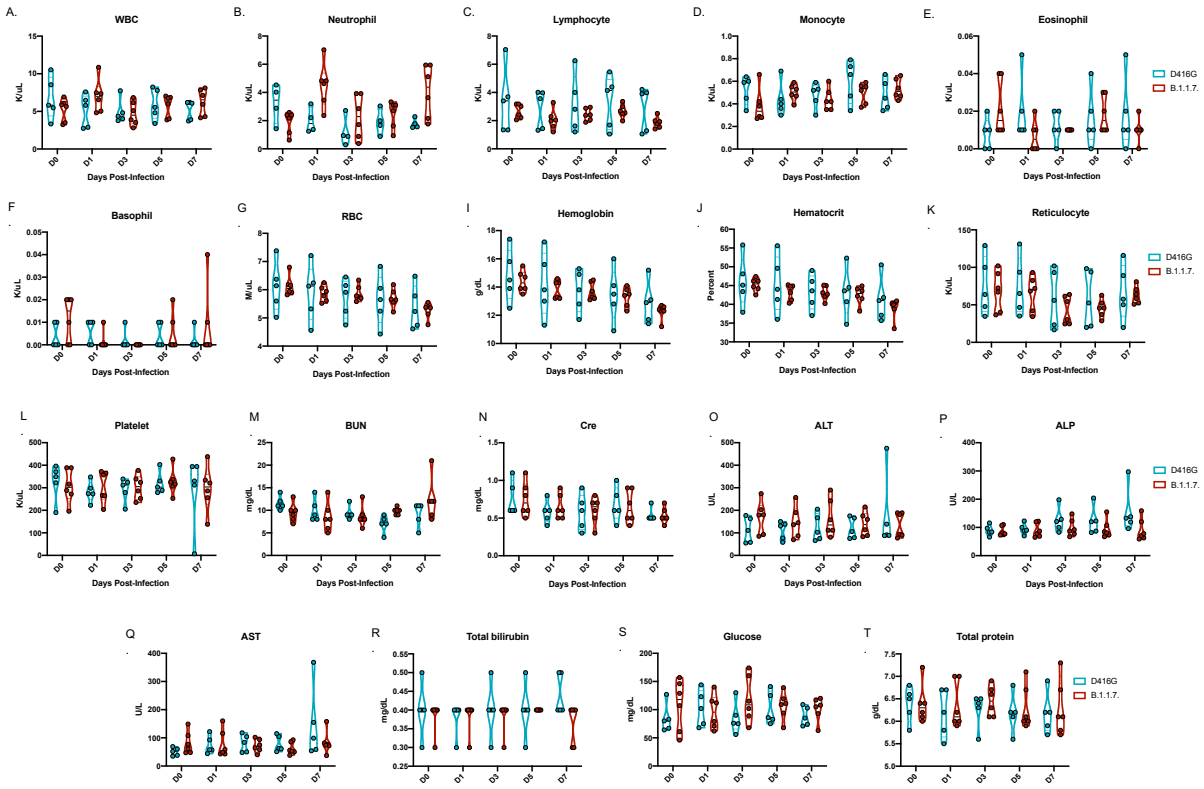

Figure S2: Hematology and blood chemistry following infection. Whole blood and serum samples were collected at each exam time point (days 0, 1, 3, 5 and 7) for hematology (A-L) and blood chemistry analyses (M-T). No significant changes were found in hematology (A-L), nor in blood chemistry (M-T).

Fig S3

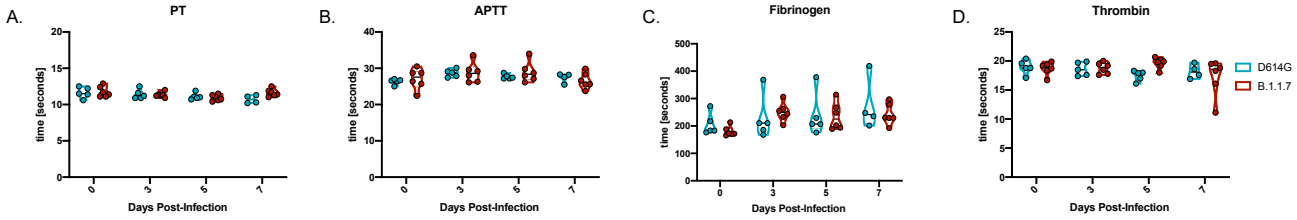

Figure S3: Coagulation assays following infection. Plasma samples were collected at each clinical time point (days 0, 1, 3, 5 and 7) to evaluate coagulation parameters between infected animals (A-D). No significant changes were found in PT (A), APTT (B), fibrinogen (C) or thrombin (D).

Fig S4

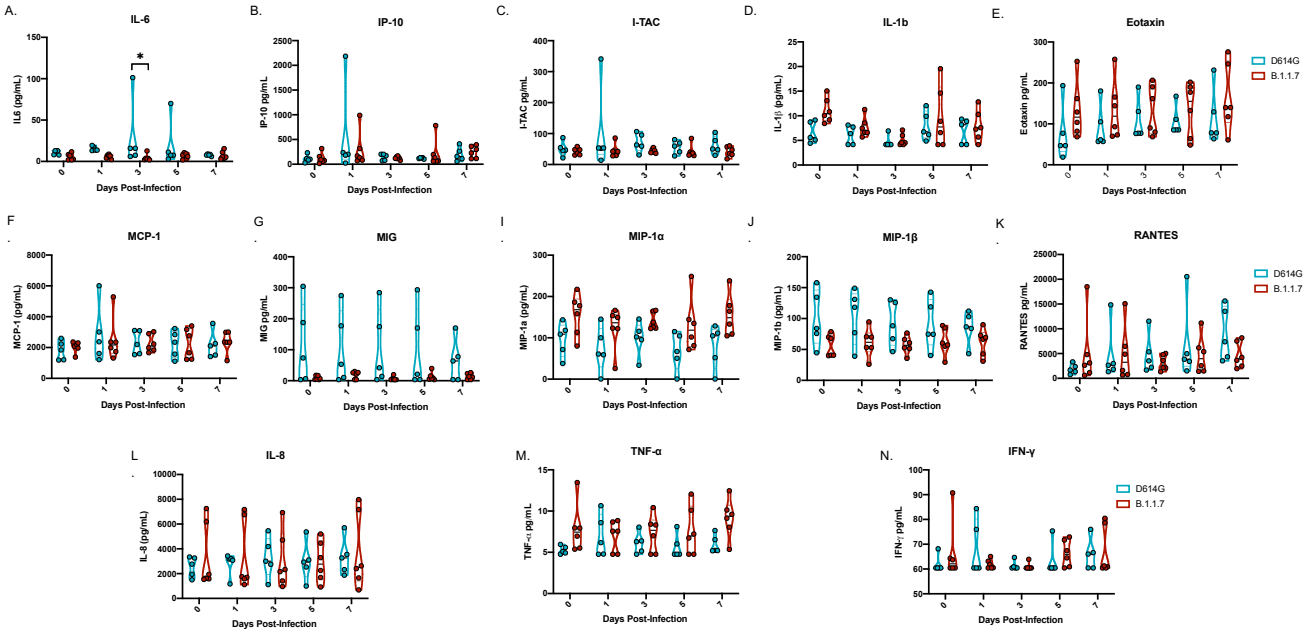

Figure S4: Cytokine analyses following infection. Serum was collected on days 0, 1, 3, 5 post-infection for cytokine analyses. Three notable changes were detected. Levels of IL-6 were significantly different 3 days post-infection between the two groups (*p-value 0.05) (A). Differences at 1 day post-infection were noted in both IP-10 (B) and I-TAC (C) but were not significant. Samples were analyzed by 2-way ANOVA to determine significance.

Table S1

	ID	Day 0-3	Day 4-7	Necropsy notes
D614G	CoV 499	Clear nasal discharge, reduced appetite Score:0/8/5/5	Severely reduced appetite Score: 5/5/5/5	Lung weight: 38.34g/animal 6.70kg RML dorsal 10%;10% ventral; accessory lobe ventral 30%; LML 30% ventral, LLL dorsal 5%, ventral 10%, Peritoneal cavity: fibrin tags Pleural cavity: adhesions Lung adhesions
	CoV 500	Reduced appetite Score:0/0/3/5	Reduced appetite Score:5/5/5/5	Lung weight: 26.38g/animal 4.29kg LLL ventral 10%
	CoV 501	Reduced appetite, slightly irregular respirations day 3 Score: 0/5/8/10	Reduced appetite, slightly irregular Abdominal respirations, slow, hunched posture, ruffled fur Score: 13/5/5/5	Lung weight: 21.06g/animal 3.36kg RML ventral 5%, dorsal 30% Fibrous adhesions
	CoV 502	Reduced appetite, Score: 0/3/3/0	Reduced appetite Score: 0/3/0/3	Lung weight: 24.73g/animal 4.86kg Lung FTC, adhesions, RML ventral 10%; RLL dorsal 50% Liver pale
	CoV 503	Reduced appetite, pale appearance, Score: 0/3/0/3	Reduced appetite, slightly increased abdominal respirations Score: 3/8/3/3	Lung weight:24.27g/animal 5.71kg RML ventral 10% Liver pale
B.1.1.7	CoV 504	Reduced appetite, deep abdominal respirations Score:0/6/6/6	Reduced appetite, quiet Score: 3/0/0/3	Lung weight: 23.33g/ animal 3.94kg RUL 10% dorsal, LML 10% dorsal; LLL dorsal 10%
	CoV 505	Reduced appetite, slow, irregular respirations Score: 0/6/8/6	Reduced appetite Score:3/0/0/3	Lung weight: 28.37g/animal 6.06kg RUL dorsal 10%; RML dorsal 10%, ventral 10%; RLL dorsal 20%, ventral 50%, LML dorsal 30%, ventral 20%; LLL 70% dorsal dark red
	CoV 506	Reduced appetite, increased abdominal respirations, hunched posture Score: 0/5/15/8/8	Reduced appetite, hunched posture Score: 8/8/5/8	Lung weight: 23.8g/animal 5.16kg RLL 10% ventral consolidated Liver: pale
	CoV 507	Reduced appetite, slightly irregular respirations Score: 0/6/6/6	Reduced appetite, slightly irregular respirations Score: 6/3/3/3	Lung weight: 37.86g/animal 4.91kg RUL 50% ventral; RLL dorsal % ventral 50% bright red Lung FTC Liver pale
	CoV 508	Score: 0/0/0/0	Reduced appetite, Score: 3/0/0/0	Lung weight:27.59g/animal 4.87kg RUL 10% dorsal; RLL 20% dorsal, Liver pale
	CoV 509	Reduced appetite, slow & tired Score: 0/5/8/5	Slow & tired score: 5/5/0/0	Lung weight: 20.83g/animal 4.01kg RLL 10% dorsal, 20% ventral bright red Liver pale

Table S1: Clinical scoring and necropsy notes of infected animals. AGMs were scored daily for clinical signs of disease including changes in general appearance, respiration, food intake, fecal output as well as locomotion. Macroscopic scoring of organs was performed during necropsies (day 7 post-infection).

Table S2

Variant	Animal	D0	D1	D3	D5	D7
D614G	499	0	1	1	1	1
	500	0	0	0	0	1
	501	0	1	1	1	1
	502	0	0	0	0	1
	503	0	1	2	2	2
B.1.1.7	504	0	2	1	1	0
	505	0	2	0	1	0
	506	0	0	0	0	0
	507	0	0	1	0	1
	508	0	2	2	0	0
	509	0	1	1	3	1

Table S2: Radiographic scoring of lungs following infection. Ventro-dorsal and right/left lateral radiographs were taken on clinical exam days prior to any other procedures (e.g. bronchoalveolar lavage, nasal flush). Radiographs were evaluated and scored for the presence of pulmonary infiltrates by two board-certified clinical veterinarians according to a standard scoring system (*7*). Briefly, each lung lobe (upper left, middle left, lower left, upper right, middle right, lower right) was scored individually based on the following criteria: 0 = normal examination; 1 = mild interstitial pulmonary infiltrates; 2 = moderate interstitial pulmonary infiltrates, perhaps with partial cardiac border effacement and small areas of pulmonary consolidation (alveolar patterns and air bronchograms); and 3 = pulmonary consolidation as the primary lung pathology, seen as a progression from grade 2 lung pathology. At study completion, thoracic radiograph findings were reported as a single radiograph score for each animal on each exam day. To obtain this score, the scores assigned to each of the six lung lobes were added together and recorded as the radiograph score for each animal on each exam day. Scores can range from 0 to 18 for each animal on each exam day.

References

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