# Investigating SARS-CoV-2 Infection Via Oral Exposure in Hamsters and Viral RNA Shedding in Feces

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## Introduction

The COVID-19 pandemic is an ongoing global public health crisis caused by the SARS-CoV-2 virus. The main pathway of transmission is through exposure to respiratory droplets and particles. Following exposure, the virus infects cells by binding to ACE-2 receptors which are expressed in epithelial tissue throughout the body. A fecal-oral transmission risk may exist, as several reports have identified prolonged excretion of SARS-CoV-2 RNA from the GI tract. Although infectious virus has not yet been recovered, further investigation is required to rule out the potential risk of fecal-oral transmission. The objective of this study is to determine the duration of viral RNA shedding for up to 21 days, assess the presence of infectious virus, as well as examine pathology following oral exposure to the virus.

## Background – Previous Study Summary

- Previously, a study was executed in which hamsters were exposed to 0.8mL 1 x $10^6$ TCID50 of the UK and WA1 strains of the SARS-CoV-2 virus via oral gavage. Information on each study group is shown in the table below.

**Table 1. Study 1 Experimental Design**

| Group | Number of Hamsters | Challenge Material | Day of Sacrifice |
|---|---|---|---|
| 1 | 6 | SARS-CoV-2 WA1 Strain | 7 |
| 2 | 6 | SARS-CoV-2 UK Strain | 7 |
| 3 | 2 | PBS Control | 7 |

- Following infection, stool samples were collected over seven days and submitted for nucleotide extraction and qPCR analysis. The results of the study are summarized below.

**Figure 1. Log Copies/g vs Days Post Infection, Study 1**

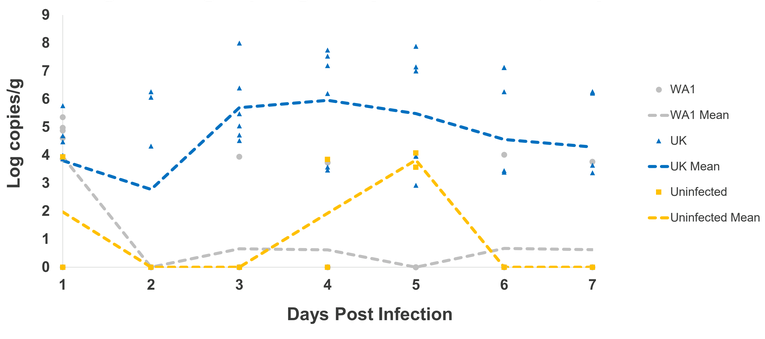


**Figure 1: Log Copies/g vs Days Post Infection;** SARS-CoV-2 UK variant established an infection in all group three animals, while WA1 fell off following the first study day. We suspect that the difference in performance was due to a mutation in the furin cleavage site in the WA1 variant virus, which would inhibit the virus' ability to bind to ACE2 receptors and establish an infection.

- In order to learn more about the duration of virus shedding, the study was repeated with additional subjects and sample collection days. The WA1 variant was also dropped from the study design.

## Experimental Design and Methods

- Hamsters were exposed to 0.8 mL 1 x $10^6$ TCID50 of SARS-CoV-2 virus (UK Variant) or PBS control on Study Day 0 via oral gavage. Additional information on the hamster study groups are shown in the table below.

**Table 2. Study 2 Experimental Design**

| Group | Number of Hamsters | Challenge Material | Day of Sacrifice |
|---|---|---|---|
| 1 | 6 | ~1 x $10^6$ TCID50 SARS-CoV-2 UK Strain | 7 |
| 2 | 6 | | 14 |
| 3 | 6 | | 21 |
| 4 | 4 | PBS Control | 7 |
| 5 | 4 | | 14 |
| 6 | 4 | | 21 |

- Each day post-infection, feces were collected and stored at < -60° C until analysis. Feces samples were then subject to TCID50 and PCR analysis.
- All animals were observed twice daily for signs of clinical illness. At the conclusion of the study nasal turbinates, lung, stomach, cecum, duodenum, colon, ileum, jejunum, and heart were collected and fixed in formalin.
- Prior to PCR analysis, samples were weighed and lysed via bead beating. Lysed samples were then extracted using the MagMAX Microbiome Extraction kit. Each extract was plated three times, with two SARS-CoV-2 target wells (N1 target) and one extraction control target well. A standard curve was used to quantitate the amount of SARS-CoV-2 in each sample. Inconclusive samples (½ SARS-CoV-2 replicates testing positive) were retested from extract once.

## Results

**Figure 2. Log Copies/g vs Days Post Infection, Study 2**

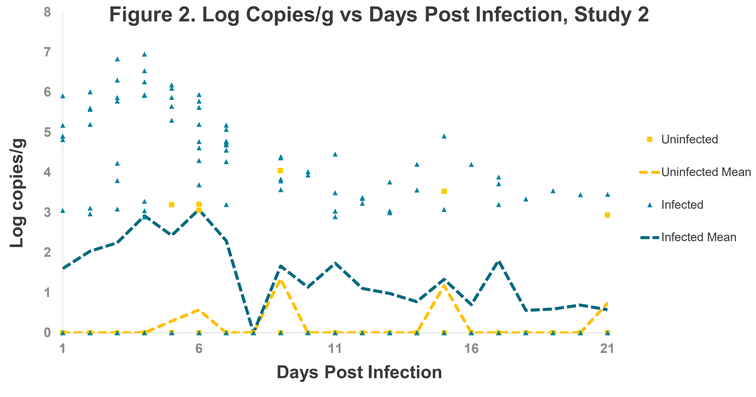


**Figure 2: Log Copies/g vs Days Post Infection;** SARS-CoV-2 shedding was observed up to 21 days post challenge day. An infection was not established in up to 39% of the animals dosed with SARS-CoV-2, resulting in a lower average trendline observed in study 2.

## Results

**Table 3. Study Incidences of Selected Microscopic Findings**

| Group | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Oral Challenge Agent | Virus | Virus | Virus | PBS | PBS | PBS |
| Termination Day | 7 | 14 | 21 | 7 | 14 | 21 |
| Number Examined[1] | 6 | 6 | 6 | 4 | 4 | 4 |
| **Lung** | | | | | | |
| Aggregate, alveolar macrophage | 4 | 3 | 5 | 0 | 0 | 1 |
| Inflammation, interstitial | 3 | 1 | 2 | 0 | 0 | 0 |
| Vasculitis | 5 | 3 | 2 | 0 | 0 | 0 |
| **Nasal Turbinate** | | | | | | |
| Exudate | 3 | 0 | 0 | 0 | 0 | 0 |
| Inflammation, olfactory epithelium | 3 | 0 | 0 | 0 | 0 | 0 |
| Inflammation, respiratory epithelium | 4 | 0 | 0 | 0 | 0 | 0 |

**Table 3:** Pathological findings show inflammation, aggregate alveolar macrophages, and vasculitis in the lungs of 50-83% of Day 7 hamsters and there was exudate and inflammation in nasal turbinates of 50-67% of the Day 7 hamsters. Only 50% of Day 14 hamsters show aggregate alveolar macrophages however they reappear in 83% of Day 21 hamsters.

**Figure 3. Percentage of Positive Results vs Days Post Infection**

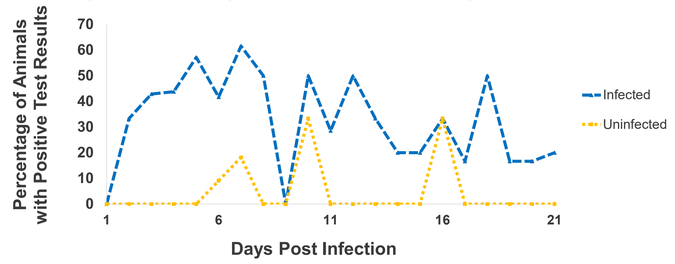


**Figure 3: Percentage of Positive Results;** 6/138 of expected negative samples (< 5%) produced false positive results. Three of the six false positives were inconclusive (1/2 SARS-CoV-2 replicates tested positive) after repeat testing.

## Discussion

- We successfully demonstrated that a SARS-CoV-2 infection can be established via the oral exposure route and observed viral RNA shedding for up to 21 days post infection.
- Although clinical symptoms resolved after approximately 7 days, the pathological findings suggests that a re-infection may be occurring, potentially due to fecal exposure.
- Results of the TCID50 assay were negative but this could potentially be due to the high limit of detection of the assay.
- Moving forward, in situ hybridization to localize the primary site of infection would allow us to assess if a subclinical infection is established in the GI-tract. Gaining an understanding of this transmission pathway will allow for a more thorough assessment of the fecal-oral transmission risk.

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