



Correspondence

Evaluation of the susceptibility of mice & hamsters to SARS-CoV-2 infection

Sir,

Coronaviruses belong to the family *Coronaviridae* with a single-stranded, positive-sense RNA genome (26-32 kb), and are known to cause infections in humans and animals. Cases of pneumonia of unknown aetiology were reported in Wuhan, China, in early December 2019, and were identified to be caused by a novel coronavirus, named as severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2)¹. As of May 27, 2020, more than 5.7 million cases were reported worldwide with above 3,52,000 deaths². As efforts are being made worldwide for the development of vaccines and antiviral drugs against SARS-CoV-2, development of animal models to study the efficacy of such control and intervention measures is also important. To address this, we evaluated the susceptibility of a few laboratory rodents to SARS-CoV-2, at the Maximum Containment Laboratory, Indian Council of Medical Research - National Institute of Virology, Pune, India.

SARS-CoV-2 utilizes angiotensin-converting enzyme 2 (ACE2) receptors to gain entry into epithelial cells, similar to SARS-CoV³. Considering this, the animal models were investigated for SARS-CoV-2 susceptibility. SARS-CoV replication without any clinical signs was demonstrated in inbred strains of mice such as BALB/c, C57BL6 and 129S⁴. The use of transgenic mice expressing the human ACE2 receptor, aged mice, knockout mice and use of mice adapted virus were found beneficial in recapitulating the clinical signs of SARS-CoV infection⁴⁻⁷. Golden Syrian hamster is another rodent model that supports SARS-CoV replication⁸. A recent study showed that the SARS-CoV-2 virus replicated in the respiratory and gastrointestinal tracts of golden Syrian hamsters and was transmitted efficiently to co-housed contact hamsters⁹. Non-rodent models such as ferrets and non-human primates have also been used to study SARS-CoV and SARS-CoV-2 infection, but cost and limited

availability make it difficult to conduct infection studies in these animals^{10,11}.

Laboratory mice and hamsters are advantageous due to their low cost, small size and availability. They can also be manipulated at the genetic level, and immunological reagents are available to study viral pathogenesis. Currently, there are no studies on mice regarding the susceptibility to SARS-CoV-2 except one on the transgenic mice with hACE2¹². Therefore, we studied the susceptibility of rodent models such as BALB/c mice, C57BL/6 mice and golden Syrian hamsters to the SARS-CoV-2 infection. All the experiments were performed with the prior permission of the Institutional Animal Ethics Committee and Institutional Biosafety Committee, ICMR-NIV, Pune.

SARS-CoV-2 isolated from throat swab sample of a human patient having a tissue culture infective dose 50 (TCID₅₀) titre of 10^{6.57}/ml was used in the study¹³. The experiments were performed in the Maximum Containment Laboratory, ICMR-NIV, Pune. Twenty adult (6-8 wk old) female BALB/c and C57BL/6 mice and 21 (two months old) Syrian hamsters were used for the study. The animals were housed in individual ventilated cages. Sixteen mice (BALB/c and C56BL/7) and 18 hamsters were inoculated by intranasal route with 5×10^{4.5} and 1×10^{5.5} TCID₅₀ SARS-CoV-2, respectively, under brief isoflurane anaesthesia. The animals were monitored daily for any clinical signs. Body weight was also monitored every alternate day. Four mice each were sacrificed on days 1, 3, 5 and 21 post inoculation and three hamsters each were sacrificed on days 3, 5, 7, 10, 14 and 21. Blood, rectal swab, oropharyngeal swab, conjunctival swab, nasal wash, nasal turbinates, lungs, heart, liver, kidney, spleen and intestine were collected. The swab samples and weighed organ samples were collected in sterile tissue culture medium (Gibco Minimum Essential Media, Thermo Fisher Scientific, USA). The organ samples were lysed using

a tissue homogenizer (Qiagen, Germany). Viral RNA was extracted from the samples using MagMax™ RNA Isolation Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) was performed¹⁴. Anti-SARS-CoV-2 IgG antibody response was assessed by in-house developed direct ELISA and neutralizing antibody levels by microneutralization test standardized at the laboratory.

No apparent clinical signs or mortality was observed in BALB/c mice, C57BL/6 mice and Syrian hamsters inoculated with the virus. The body weights of hamsters reduced post virus inoculation (Fig. 1). Progressive weight loss following SARS-CoV-2 infection in hamsters has been reported⁷. The weight loss ranged from 1.4 to 6.7 per cent on day 2 (n=18), which reached to a maximum on day 6 ranging from 5.1 to 13.4 per cent (n=12). Thereafter, a gradual weight gain was observed in hamsters on the subsequent days.

Viraemia was absent in both mice and Syrian hamsters. Viral RNA could be detected only in the lung samples collected on day 1 post inoculation in BALB/c and C57BL/6 mice, and the lung samples collected on subsequent days and rest of the organ samples from mice were found negative. The viral RNA detected in the lungs could be the residual RNA from the virus inoculums. The BALB/c and C57BL/6 mice were found negative for anti-SARS-CoV-2 IgG antibodies by direct ELISA on day 21, indicating that these adult inbred mice were not susceptible to SARS-CoV-2. The animal models studied showed the similar findings of absence of virus in blood samples as reported in COVID-19-positive patients¹⁵.

In Syrian hamsters, the viral RNA could be detected in nasal turbinates, trachea, lungs, spleen and

kidney on day 3 post inoculation. The highest viral load (mean viral RNA copy number) was detected in lungs (1.6×10^{10}), followed by trachea (5.3×10^9), nasal turbinates (4.6×10^9), kidney (8.4×10^6) and intestine (3.3×10^5) on the third day (Fig. 2). This shows resemblance to the upper and lower respiratory tract affinity of SARS-CoV-2 in humans and the higher viral loads observed during the acute phase of infection¹⁶. The reduction in the viral load was observed in the organs in the subsequent days, and complete clearance was observed from the small intestine by day 5, kidney by day 7 and trachea by day 10). The viral RNA persisted in the lungs and nasal turbinates till days 14 and 21, respectively. This observation was in line with a recent study that reported viral shedding up to a median period of 20 days in COVID-19 survivors¹⁵. The high viral RNA load could be detected in nasal wash and oropharyngeal swab till day 7. In contrast to the human studies which showed viral RNA detection in anal swabs¹⁷, the rectal swabs collected at various time points in hamsters were negative for viral RNA although intestine samples tested positive till day 5. The conjunctival swab samples were also negative.

To assess the risk of transmission, virus isolation was attempted from the lung and nasal turbinates samples collected from hamsters on days 3, 5, 7 and 14 post inoculations. The cytopathic effect in Vero CCL81 cells could be observed only on days 3 and 5 for both lung and nasal turbinate samples. This finding supported a report on human COVID-19 cases which indicated that the transmission might occur early during the course of infection¹⁴. The serum samples of hamsters showed neutralizing antibody from day 5 onwards with rising titre till day 21. Serum IgG levels also showed a similar trend from day 7 (Fig. 3).

In conclusion, our findings indicated the susceptibility of a readily available hamster model to SARS-CoV-2 infection. Golden Syrian hamsters showed high viral loads in the upper and lower respiratory tracts, virus shedding through the nasal cavity and mounting of humoral immune response by the first week, similar to human COVID-19 cases.

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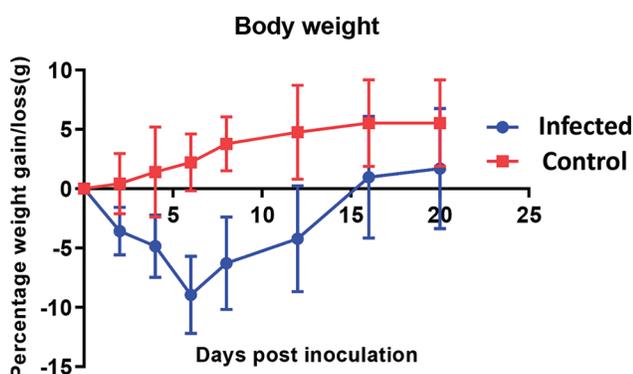


Fig. 1. Percentage weight gain/loss (mean \pm standard deviation) at day 2 (n=18), day 4 (n=15), day 6 (n=12), day 8 (n=9), day 12 (n=6), day 16 (n=3), day 20 (n=3) of hamsters following intranasal SARS-CoV-2 virus inoculation and control hamsters (n=3).

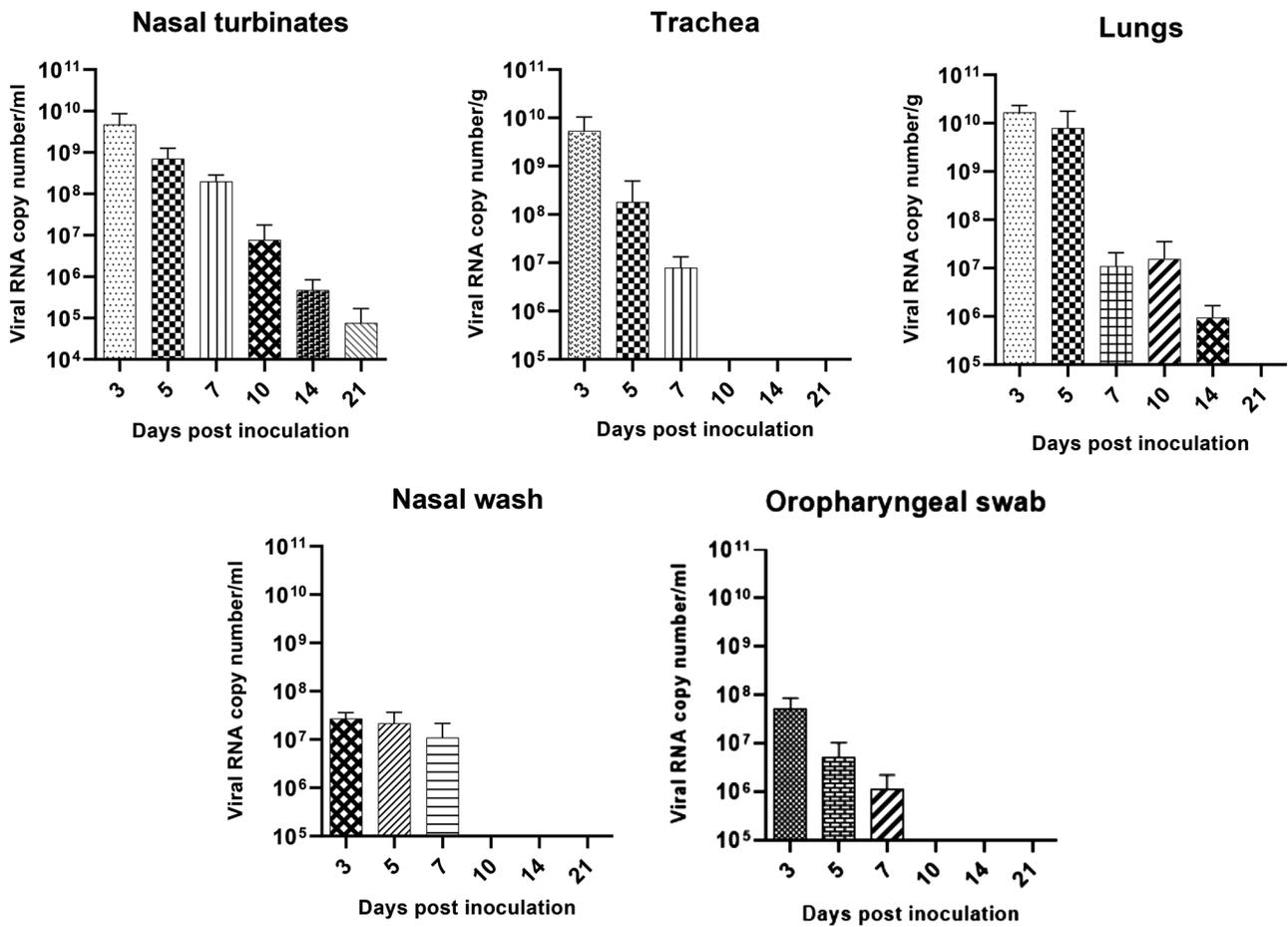


Fig. 2. SARS-CoV-2 viral RNA copy number (mean ± standard deviation, n=3) in the nasal turbinates, trachea, lungs, nasal wash and oropharyngeal swabs of hamsters.

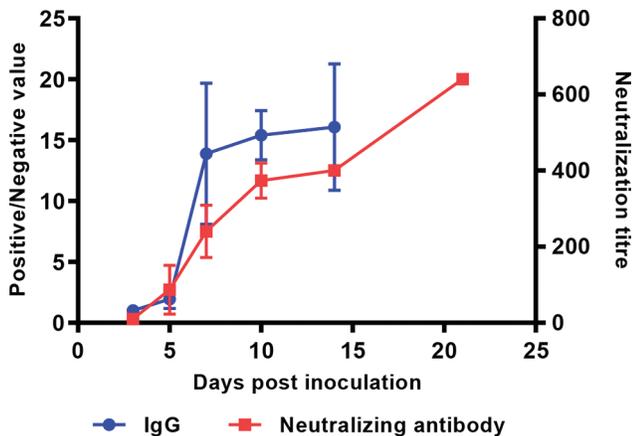


Fig. 3. Anti-SARS-CoV-2 IgG levels (Positive/Negative value in direct ELISA) and neutralizing antibody titre (mean ± standard deviation, n=3) in hamsters post virus inoculation.

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