



Letter

Infection and pathogenesis of the Delta variant of SARS-CoV-2 in *Rhesus macaque*

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Dear Editor,

COVID-19 caused by SARS-CoV-2, is still a big threat to human populations around the world. As of Jan 2022, over 292 million cases were reported worldwide with more than 5.4 million deaths. After it was first reported in China in December 2019, the virus kept circulating and evolving and several variants with different transmissibility emerged in different countries and regions (WHO, 2021a). Until now, six major variants have been reported including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Lambda (C.37), and Omicron (B.1.1.529) and the infection caused by different variants has varied according to the surveillance data (WHO, 2021b).

The Delta variant was firstly identified in India in Dec 2020 and the World Health Organization (WHO) declared it a “variant of concern (VOC)”, since it was shown to be more contagious, more deadly and more resistant to the current vaccines and treatments (WHO, 2021b). During May to the beginning of December 2021, Delta variant was the dominant circulating strain in most regions of the world, with around 60% greater transmissibility than the Alpha variant, 1000 times higher viral load than the strains in the initial epidemic wave of 2020, an increased 2.26-fold risk of hospitalization compared with Alpha and was moderately resistant at a degree of around 30.7% to vaccines BNT162b2 or ChAdOx1 nCoV-19 particularly in people with a single dose (Bian et al., 2021; Callaway, 2021; Planas et al., 2021; Shi and Dong, 2021). The proliferation of Delta variant worldwide poses huge challenges for global pandemic control, particularly in those countries and regions that have limited access to vaccines.

Previous studies have shown that SARS-CoV-2 replicates to a high viral load in the respiratory tract of *Rhesus macaques* and causes pneumonia utilized for studying pathology, immune response, and multiple preclinical drug and vaccine evaluations (Chandrashekar et al., 2021, 2020; Deng et al., 2020; Feng et al., 2020; Mercado et al., 2020; Shan et al., 2020). This model is important for generating information on disease characteristics and replicative fitness and for evaluating the pharmaceutical treatment approaches against the new variant.

In the present study, pathogenicity and virus shedding of the Delta variant in rhesus macaques were assessed. The shedding pattern and pathogenesis changes were like that observed in our prior studies, but with rapid and strong replication in macaques when compared to the early SARS-CoV-2 strains during the first epidemic that occurred in humans, in Wuhan, China in late 2019 (Shan et al., 2020).

Three adult rhesus macaques aged from six to eleven years, were inoculated with a total of 1×10^6 TCID₅₀/mL of Delta variant (B.1.617.2) administered as 1 mL volume by the intratracheal (IT) route as high dose (HD) group (Fig. 1A). And three were inoculated with 1×10^5 TCID₅₀/mL as low dose (LD) group. Animals exhibited slightly decreased appetite and responsiveness suggestive of mild clinical disease, as well as mild weight loss in the high-dose group. Fever, respiratory distress and mortality were not observed.

RNA extraction and RT-qPCR detection for SARS-CoV-2 from swabs, blood, and tissues as described previously (Shan et al., 2020). Elevated levels of viral RNA in the throat were observed with a median peak of 6.7 (range 6.0–7.3) log₁₀ and 7.6 (range 3.4–7.9) log₁₀ RNA copies/swab in HD and LD groups, respectively (Fig. 1B). No significant difference was found regardless of dose. Much lower virus load was found in the nasal

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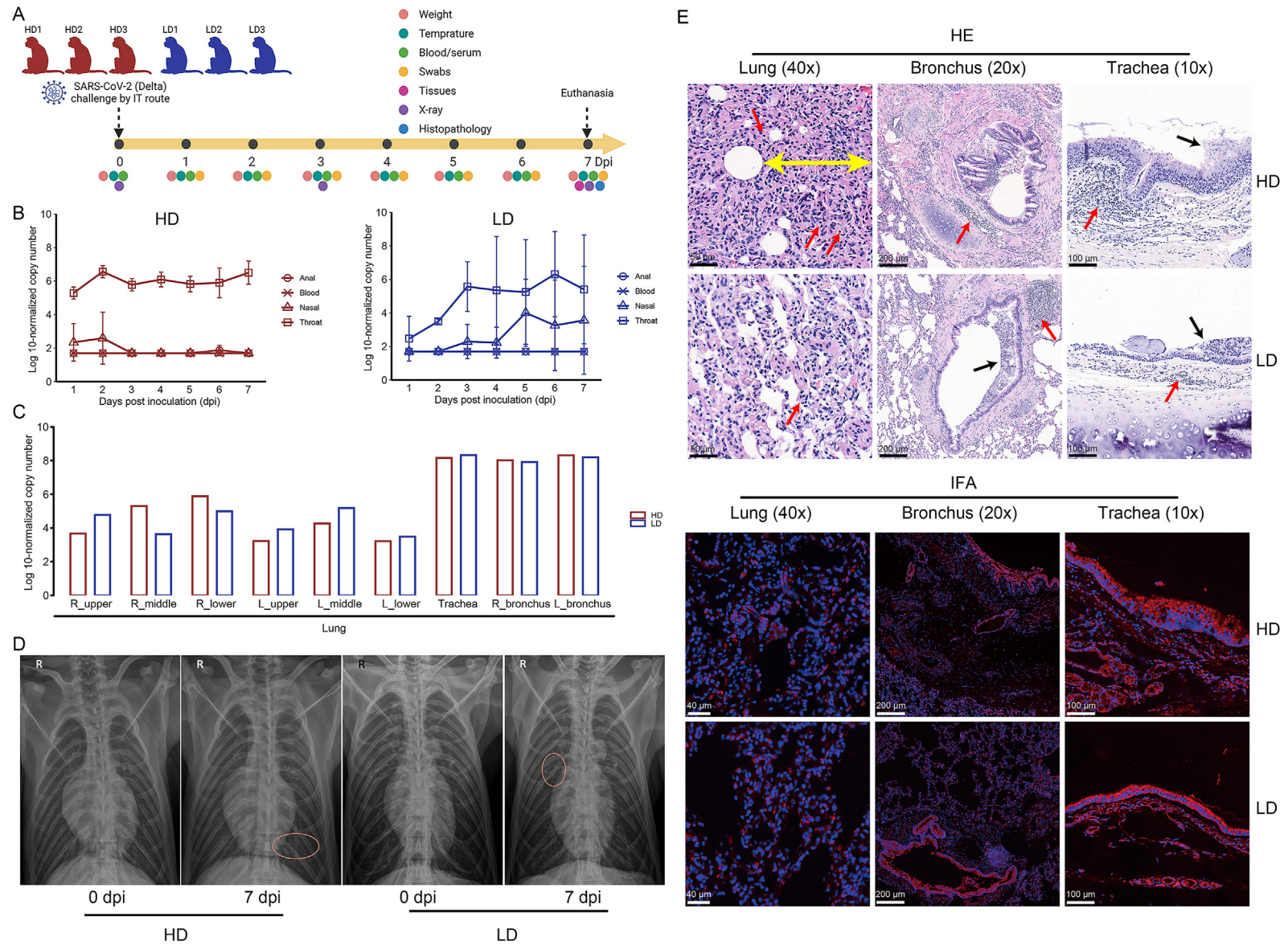


Fig. 1. Experimental scheme, viral RNA load, thoracic X-rays, histopathological and immunological fluorescence assay in SARS-CoV-2 Delta variant infected rhesus macaques. **A** Experimental scheme and experimental parameters. **B** Viral load in swabs and blood of the Delta variant infected rhesus macaque. Viral load (mean $\pm$ SEM) of anal, blood, nasal and throat specimens collected from the inoculated macaques (HD, $n = 3$; LD, $n = 3$) on 1 to 7 dpi. **C** Viral loads in lung of the Delta variant infected rhesus macaque. Varied samples including lobe of lung tissue, bronchus and trachea collected from inoculated macaques (HD, $n = 3$; LD, $n = 3$) at 7 dpi. **D** Anterior-posterior thoracic X-rays of infected rhesus macaque. The images have been taken at 0 and 7 dpi of HD and LD macaques. Areas of interstitial infiltration, indicative of pneumonia are highlighted with red ellipse. **E** Histopathological and immunological fluorescence assay (IFA) observation of the Delta variant infected rhesus macaque. Yellow arrows indicate alveolar wall thickening, black arrows indicate the epithelial cells shedding, red arrows indicate the inflammation cells in HE staining. Red color indicates nucleocapsid protein (NP), and blue color indicated the cell nuclei in IFA. Lung, bronchus, and trachea tissue were collected at 7 dpi from HD and LD macaques. HD, high dose; LD, low dose; dpi, days post inoculation; SEM, standard error of mean.

specimens. No viral RNA was detected in blood and anal samples (Fig. 1B). Viral RNA in the throat increased in all animals in HD and LD groups from days one to two, suggesting viral replication.

All macaques in both groups were euthanized and necropsied at 7 days post inoculation (dpi). Different lobes of lung, trachea, and bronchus were collected and viral RNA was detected in all tissues. Much higher viral load was presented in the trachea and bronchus when compared to lung, with a median value of 8.2 (range 6.8–8.6) log₁₀ and 8.2 (range 4.1–8.6) log₁₀ RNA copies/g in trachea and a median value of 8.1 (range 5.2–8.6) log₁₀ and 8.1 (range 3.8–8.6) log₁₀ RNA copies/g in the bronchus (Fig. 1C).

The anterior-posterior thoracic X-rays of macaques from both of the HD and LD groups showed intense patchy opacity in the middle and lower parts of lung lobes at 7 dpi (Fig. 1D). For the histopathological analysis and SARS-CoV-2 antigen detection, tissues from lung, bronchus, and trachea were fixed, then hematoxylin-eosin (HE) stain, immunological fluorescence assay (IFA), and immunohistochemical (IHC) were conducted separately as described previously (Shan et al., 2020). Microscopically, lesions were in the lungs where the interstitial pneumonia present was severe. Pneumonia from LD macaques was characterized by fibrosis and organization of some alveolar wall and alveolar cavity, mild to moderate thickening of alveolar walls, increased numbers of monocytes, inflammatory cell infiltration including lymphocytes and monocytes and fibroblast proliferation. The HD macaques exhibited diffuse, severe interstitial pneumonia along with multiple massive pulmonary consolidation and extensive fibrosis and organization of alveolar cavity and alveolar wall, also accompanied by massive of fibroblast proliferation, monocyte and lymphocyte infiltration. Some alveolar walls showed increased macrophages and protein edema exudation, hyaline membrane formation and occasionally multinucleate giant cells while hyaline thrombus was occasionally seen in alveolar capillaries. In the bronchus, the bronchial epithelial cells were lost and quantities of mucus, shed epithelial cells, monocytes and lymphocytes were observed in the lumen. Monocytes and lymphocyte infiltration can be seen in the wall of the small bronchial tubes (Fig. 1E).

IFA indicated viral nucleocapsid protein (NP) was detected in the lung, bronchus and trachea of all infected macaques which showed as strongly positive in trachea and bronchus (Fig. 1E). IHC analysis of HD and LD macaques by SARS-CoV-2 NP antigen revealed that the positivity was distributed in pulmonary macrophages around the alveolar wall, bronchial mucosa epithelium, goblet cells and pseudostratified ciliated epithelial cells of the tracheal mucosa epithelium, lymphocytes of the mucosal lamina propria and tracheal submucosal glands (Supplementary Fig. S1).

Even the infection dose for Delta variant in this experiment was 1×10^6 TCID₅₀ or 1×10^5 TCID₅₀ per animal, which was 7 or 70 folds lower than the early strain IVCAS 6.7512 (at 7×10^6 TCID₅₀ per animal) used in our previous study, high viral load in throat and severe pathogenic changes in lung were still be observed (Shan et al., 2020). The viral RNA load in throat of infected animal with Delta variant took 2–3 days to reach peak while the early strain took around 5 days (Shan et al., 2020), supporting the attributed disease rapid spread of the Delta variant. In addition, whether infected with early strain or Delta variant, no significant changes in the body temperature, weight loss, blood cell counts (Supplementary Fig. S2) and X-ray imaging were detected, which might be due to the virus could only cause asymptomatic or mild clinical symptoms in macaque (Shan et al., 2020). This suggests that the viral load in the throat and the pathogenic changes of lung can be used as the two main parameters for evaluating the SARS-CoV-2 infection in the rhesus macaque model.

Since the Omicron variant rapidly became a dominant strain in many countries, it has raised concerns for its transmissibility and pathogenicity. Fast spreading and decreased severity of disease in patients during the first wave of Omicron variant in South Africa was observed (Abdullah et al., 2022). In addition, the animal experiment data showed attenuated lung diseases in rodent when infected with several different Omicron isolates (Diamond et al., 2021). For now, no data from non-human

primate model of Omicron is published yet. Further evaluation and comparison for Delta and Omicron variant pathogenesis and vaccine efficacy should be done.

Footnotes

This study was financially supported by the STS regional key project (KFJ-STSYZD-2021-12-001 to Z.M.Y. and C.S.) from Chinese Academy of Sciences, the National Key R&D Program of China (2021YFC0863300 to Z.M.Y. and C.S.), the National Key R&D Program of China (2021YFE0201900 to C.S.), and the Science and Technology Major project of Hubei Province (2021ACB005 to C.S. and H.X.). We are particularly grateful to the staff at the Wuhan National Biosafety Laboratory of the Chinese Academy of Sciences. We thank the Center for Instrumental Analysis and Metrology and BSL-3 laboratory, Wuhan Institute of Virology. We also thank National Virus Resource Center, Wuhan Institute of Virology, CAS and National Pathogen Resource Center, NRPC for providing Delta strain (Strain No.: CSTR.16698.06.NRPC.6.CCPM-B-V-049-2105-8). No potential conflict of interest was reported by the author(s). The procedures for animal care and use are followed associated legislation and guidelines, and the study is approved by the Committee of Wuhan Institute of Virology, Chinese Academy of Sciences (Approval No. WIVA42202002-01).

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.virs.2022.02.001>.

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