Protective efficacy of COVAXIN® against Delta and Omicron variants in hamster model

Highlights
COVAXIN immunization protected hamsters against Delta variant infection
Pneumonia severity was considerably reduced in three-dose-immunized hamsters
Summary

The immunity acquired after natural infection or vaccinations against SARS-CoV-2 tend to wane with time. Here, we compared the protective efficacy of COVAXIN® following two- and three-dose immunizations against the Delta variant and also studied the efficacy of COVAXIN® against Omicron variants in a Syrian hamster model. Despite the comparable neutralizing antibody response against the homologous vaccine strain in both the two-dose and three-dose immunized groups, considerable reduction in the lung disease severity was observed in the 3 dose immunized group after Delta variant challenge. In the challenge study using the Omicron variants, i.e., BA.1.1 and BA.2, lesser virus shedding, lung viral load and lung disease severity were observed in the immunized groups. The present study shows that administration of COVAXIN® booster dose will enhance the vaccine effectiveness against the Delta variant infection and give protection against the BA.1.1 and BA.2 variants.

Introduction

SARS-CoV-2 has evolved to numerous variants in the last two years posing a challenge to mankind. The immunity generated by the natural infection or vaccination tends to wane with time (Centre for Disease Control and Prevention.CDC, 2020; Feikin et al., 2022). Waning of immune responses after primary two-dose series of vaccinations has been reported for the available COVID-19 vaccines (Khoury et al., 2021). The newly evolving variants have also posed challenges to the acquired immunity with their immune escape properties. Thus, the Variants of Concerns (VoCs) which have evolved until now managed to spread globally even after good vaccination coverage. Vaccine break through infections are being reported worldwide (Kirtan et al., 2021). This necessitates the importance of constant monitoring of the virus genomic changes and the properties of the evolving variants. The decline of immunity varies with vaccine products and the target vaccination group. The World Health Organization has recommended the administration of an additional dose for inactivated COVID-19 vaccines like CoronaVac and BBIP (World Health Organization, 2021). Booster doses have shown to improve the efficacies of many COVID-19 vaccines in clinical trials and have been authorized by regulatory authorities in many countries (Andrews et al., 2022; Barda et al., 2021; Moreira et al., 2022; Vadrevu et al., 2022).

Vaccine effectiveness differs based on disease severity, symptomatic diseases and infections for the different VoCs (World Health Organization, 2022a). In case of the Alpha variant, protection was retained against the above-mentioned outcomes by the vaccines. With the Beta and Delta variants, the vaccines showed reduced protection against symptomatic disease whereas disease severity was found reduced in the vaccinated population. Of the 5 designated VoCs, the Omicron variant is in current circulation throughout the world and the other variants constitutes less than 1% in prevalence. The Omicron lineage has been further divided into multiple sub lineages of which a few such as BA.1, BA.2, BA.4, and BA.5 have shown growth advantage. The Omicron variant is known to escape many therapeutic/vaccine elicited neutralizing antibodies and has shown reduced effectiveness following the two-dose regimen of many COVID-19 vaccines (Planas et al., 2022; World Health Organization, 2022b).

COVAXIN® is an inactivated SARS-CoV-2 whole virion vaccine licensed in India and 23 other countries (World Health Organization, 2022c). The vaccine has shown immunogenicity and protective efficacy against
Figure 1. Study design, antibody response and body weight changes in hamsters following COVAXIN® immunization and Delta variant virus challenge

(A) Summary of the study design of protective efficacy of COVAXIN® against Delta variant infection. Antibody response in COVAXIN® immunized hamsters two weeks post the last dose of vaccination as measured by (B) Anti-SARS-CoV-2 IgG ELISA and (C) Anti-SARS-CoV-2 RBD IgG ELISA. Mean and standard deviation are plotted on the graph, n = 20/group. The dotted lines indicate the limit of detection of the assay. (D) Neutralizing antibody response in hamsters against the SARS-CoV-2 B.1 variant before Delta virus challenge (n = 10/group) and (E) after Delta variant challenge on 7 DPI (n = 5/group). The dotted lines indicate the limit of detection of the assay. Geometric mean and the standard deviation are plotted on the graph. The significant differences between the experimental groups were assessed using Mann-Whitney test and the p value is plotted above the bars of the respective groups. p values less than 0.05 were considered statistically significant.
Figure 1. Continued
(F) The mean body weight change in hamsters after Delta variant challenge on 3 (n = 10/group) and 7 DPI (n = 5/group). The error bars represent the standard deviation. The significant differences between the experimental groups were assessed using Mann-Whitney test. p values less than 0.05 were considered statistically significant.

Antibody response in hamsters after vaccination and Delta variant challenge
In the present study, we compared the protective efficacy of two and three doses of COVAXIN® against the SARS-CoV-2 Delta variant in hamsters (Figure 1A). In all the immunized animals, 14 days after the second or third dose, seroconversion was observed (Figure 1B). Anti-RBD IgG levels [mean Optical Density (OD) ± Standard Deviation (SD) = 1.27 ± 0.60 in three dose group, 0.77 ± 0.42 in two dose group, serum dilution: 1: 100] and geometric mean PRNT titers (GMT) (GM ± SD = 604.5 ± 2.4 in two dose group and 944.5 ± 4.05 in three dose group) were higher in the three-dose immunized group (Figures 1C and 1D).

The GMT against the vaccine strain after Delta variant challenge showed a 4.29-fold rise in two-dose group (GM ± SD = 2594 ± 3.32) and a 6.79-fold rise in the three-dose group (GM ± SD = 6420 ± 1.13) when compared with the titers before challenge in the immunized group. The immunized animals showed a significant rise in the protective antibody titers in comparison with the placebo group too at 7 DPI (Figure 1E). The GMT against the BBV152 vaccine strain, i.e., the B.1 variant was 123-fold and 41-fold higher in the two- and three-dose vaccinated groups, respectively. Similarly, against the Delta variant, GMT was 250 and 15 times higher than placebo in the two- and three-dose vaccinated groups, respectively. The neutralizing antibody levels were comparable against both the Delta and the ancestral B.1 variant.

Protection against SARS-CoV-2 Delta variant challenge
There was no significant change in the mean body weight loss observed in the two-dose and three-dose immunized groups with their respective controls on 3 and 7 DPI by Mann-Whitney test (Figure 1F). Area under the curve for serial measurements of body weights was calculated for each group after the challenge and was compared with placebo as well as between immunized groups and no significant difference could be observed. Three-dose immunizations did not prevent the body weight reduction in Delta challenged animals unlike the two-dose. The viral RNA shed through the throat and nasal cavity was found markedly reduced in two/three-dose vaccinated animals in comparison with the placebo groups (Figures 2A–2D). By 7DPI, viral RNA clearance from the throat swab and nasal wash was observed in the vaccinated groups. The viral loads in the nasal wash were also lower in the vaccinated groups, although a significant difference could only be seen on 3 and 7 DPI of the two-dose group (Figure 2E). When the vaccinated groups were
compared, significant viral RNA reduction was observed on 1 and 5 DPI in the throat swab and nasal wash specimens of the three-dose group.

In the nasal turbinates samples, the viral RNA and subgenomic (sg) RNAs were significantly reduced on 3 DPI, whereas sgRNA levels were similar on the 7DPI in both the two- and three-dose vaccinated groups in comparison to placebo. When the vaccinated groups were compared, the viral RNA was found significantly reduced on 3 DPI in the nasal turbinates of the three-dose group. On virus titration, the virus titers in the three-dose vaccinated group were found reduced on 3 and 7 DPI, whereas the two-dose group showed similar viral titers as that of the placebo group. Lungs samples collected on 3 and 7 DPI showed significant reduction in the viral RNA and live virus titers in the vaccinated groups in comparison with the placebo group (Figures 3D–3F). When the two- and three-dose vaccinated groups were compared, a significant reduction was seen in sgRNA levels in the lungs of the three-dose group on 3 DPI.

Histopathological changes such as perivascular and peribronchiolar inflammatory cell infiltration, alveolar hemorrhages, pneumocyte hyperplasia, and septal thickening were observed in the Delta variant challenged animals (Figures 4A and 4B). Lesions of lesser severity were observed in the two-dose vaccinated groups compared to the placebo group (Figures 4C and 4D). In the three-dose immunized group, histopathological changes observed were minimal with focal inflammatory cell infiltration or alveolar damage compared with the placebo group (Figures 4E–4H and 4J). The cumulative histopathology score were significantly lower in the three-dose immunized groups in comparison to the two-dose and placebo groups (Figure 4I and Table S1).

Protection against Omicron variant challenge

We performed a virus challenge study using the Omicron variants, i.e., BA.1.1 and BA.2, in hamsters following three-dose immunization (Figure 5A). The placebo group infected with BA.1.1 showed a mean...
weight loss of $-18 \pm 2.5$ and weight loss was not observed in the BA.2 infected hamsters of both the vaccinated and placebo groups (Figure 5B). The immunized animals showed anti-SARS-CoV-2 and anti-RBD IgG response as well as high neutralizing antibody titers against B.1 (vaccine strain) variant (GMT $\pm$ SD = 2704 $\pm$ 1.618) before virus challenge (Figure S2). The body weight loss was minimal in COVAXIN® immunized hamsters infected with BA.1.1 and BA.2 (Figure 5B).

The neutralizing antibody response against BA.1.1 was less in comparison with the BA.2 in both experimental groups (Figure 5C). The GMT was 52.92 $\pm$ 2.14 and 215.4 $\pm$ 2.67 in the BA.1.1 and BA.2 infected group against the BA.1.1 variant on 7DPI respectively. The GMTs were comparable in the immunized and placebo groups on 7 DPI against BA.1.1 variant. GMT was 41.14 $\pm$ 2.69 and 296.9 $\pm$ 1.96 in the vaccinated groups against BA.2 variant after BA.1.1 and BA.2 infection (Figure 5C). The virus shedding through nasal and oral cavity was significantly reduced in the vaccinated groups (Figure 6). Viral RNA and sgRNA levels in the nasal turbinates and lungs were also significantly reduced in the immunized hamsters after Omicron infection on days 3 and 7 after virus infection. Viral load in the nasal turbinates were comparable on 3 and 7 DPI whereas the titers in the lungs were significantly reduced in the BA.2 infected vaccinated groups on both the time points (Figure 7).

Grossly, the BA.1.1 infected hamster lungs showed severe congestion and hemorrhages and gross lung lesions were more focal in case of BA.2 infection (Figure S1). Alveolar interstitial pneumonia with peribronchial infiltration was observed in the hamsters of the placebo group infected with Omicron variants, BA.1.1 and BA.2. In the vaccinated groups, lesions observed were mostly focal in distribution (Figures 8A–8D and 8F). Mean scores of 4.37 ($\pm$ 0.478) and 2.25 ($\pm$ 0.736) were observed in the placebo and the vaccinated groups infected with BA.1.1 respectively (Figure 8E). Lesion severity following the
BA.2 infection was found to be lesser in the vaccinated hamsters with a mean (±SD) cumulative score of 2.438 (±0.47) against a score of 3.18 (±1.23) in the placebo groups. The histopathological scoring is given as supplementary data (Table S1).

**DISCUSSION**

We compared the protective efficacy of COVAXIN® following two- and three-dose immunizations against the Delta variant and also studied the efficacy of COVAXIN® against Omicron variants in hamster model. In the Delta infection study, where we compared the protective response between the two- and three-dose regimens, we could observe the advantage of the booster dose vaccination in the reducing the lung
disease severity. Although the neutralizing antibody levels were comparable among the groups, lung disease severity was found more reduced after the three-dose vaccination. Virus shedding and viral organ loads were considerably reduced in both two-dose and three-dose immunized animals indicating the vaccine efficacy against Delta variant. The difference in the antibody response could be probably because of the individual animal variation in response as the animals in the two-dose and three-dose groups were different. This also points to the role of other components of immune system in protection. We have not assessed the cellular immune response in the present study because of the limitation of availability of reagents specific for the Syrian hamsters. COVAXIN® was found to induce robust immune memory and Th1 skewed response in previous studies (Ganneru et al., 2021; Vikkurthi et al., 2021). The vaccine was also found to elicit a substantial fraction of T follicular cells which in turn can aid in long term humoral immunity (Vikkurthi et al., 2021). The proportion of the antibody secreting memory B cells in the 3 dose COVAXIN® recipients were found more compared to the two-dose recipients (Vadrevu et al., 2022).

For COVAXIN®, an effectiveness of 69% has been reported against severe COVID-19 and 50% against symptomatic COVID-19 for Delta variant in humans after two-dose vaccinations (Bhatnagar et al., 2022). The reduced effectiveness of vaccine for prevention of breakthrough infection is also demonstrated (Behera et al., 2022). These studies were performed after a two-dose vaccine regimen. Decline of neutralizing antibody response was observed 6 months after the second dose of COVAXIN® immunization; however, persistent Activation Induced Marker + SARS-CoV-2 specific CD4+ and CD8+ T cell memory phenotype was observed. A third booster dose led to pronounced increase in the neutralizing response against homologous and heterologous SARS-CoV-2 variants in humans as reported in a double-blind, randomized controlled phase 2 clinical trial (Vadrevu et al., 2022). The interval between the second and third dose vaccinations was short in the current study, unlike the real life scenario, where booster doses are

Figure 5. Study design, body weight changes and antibody response in hamsters following Omicron variant virus challenge

(A) Summary of the study design of protective efficacy of COVAXIN® against Omicron variant infection.
(B) Body weight changes in hamsters after Omicron variant challenge on 3 (n = 8/group) and 7 DPI (n = 4/group). The mean and standard deviation are plotted on the graph.
(C) Neutralizing antibody response in COVAXIN® immunized hamsters after Omicron variant challenge on 7 DPI (n = 5/group) against BA.1.1 and BA.2. The dotted lines indicate the limit of detection of the assay. The geometric mean and standard deviation are plotted on the graph. The significant differences between the experimental groups were assessed using Mann Whitney test and the p value is plotted above the bars of the respective groups. p values less than 0.05 were considered statistically significant. ns = non significant.
recommended after 6 months or after considerable decline in the antibody levels. The booster dose of COVAXIN® was found to improve the neutralizing antibody response against the VoCs including Delta and the Omicron (Deshpande et al., 2022). Vaccine effectiveness reported for an inactivated COVID-19 vaccine, CoronaVac also shows that the three-dose immunization provides better protection in terms of disease outcomes (Jara et al., 2022). The current data shows that boosting of the immune response tends to improve the vaccine effectiveness for disease severity.

Vaccine efficacy data of two-dose/three-dose COVAXIN® against Omicron variant in humans is not available. Our findings demonstrate the protective response of the vaccine against the Omicron variant. Limited or no body weight loss and reduced lung disease severity was observed in the vaccinated animals. The disease severity in the COVAXIN® immunized animals were found reduced despite fewer neutralizing antibody levels following the Omicron variant challenge. The breakthrough cases sera as well as COVAXIN® booster dose vaccinee sera demonstrated better neutralizing titers against Omicron and other VOCs in comparison with the two dose vaccinee sera (Deshpande et al., 2022; Yadav et al., 2022a, 2022b). Vaccine effectiveness of 50% was reported against symptomatic and severe disease by Omicron variant within three months of the first two doses of CoronaVac and a booster dose of the vaccine improved the effectiveness (Jara et al., 2022). The vaccine effectiveness studies with the 2 dose regimen of the primary series of mRNA vaccines (Spikevax, Comirnaty), inactivated vaccine (CoronaVac), vectored vaccines (Vaxzevria, Ad26.COV2.S.) conducted in 11 countries against Omicron variant has shown reduced effectiveness for disease severity, symptomatic disease, and infection (World Health Organization, 2022b). Booster vaccination was found to considerably improve the vaccine effectiveness in these studies (World Health Organization, 2022b). Here, we have used three-dose regimen for Omicron studies considering these observations.

The protection observed might be as a result of the cellular response induced too. In humans, it is demonstrated that the magnitude of immune responses diminishes after the second dose of
COVAXIN® over time, whereas booster dose vaccinations enhance the neutralizing antibody responses against homologous and heterologous variants (Vadrevu et al., 2022; Yadav et al., 2022a, 2022b). Apart from B cell mediated immunity, the SARS-CoV-2 specific central and effector memory cells which can aid in cytotoxic function (CD8+ TEMRA phenotype) has been demonstrated in the COVAXIN® recipients (Vikkurthi et al., 2021). These properties could have attributed to the rapid reduction of viral load after challenge. Although marked difference was not observed in the histopathological cumulative score of the BA.2 infected groups, the mean scores of the vaccinated groups were considerably lower compared to our earlier studies on pathogenicity of BA.1.1 in hamsters (Mohandas et al., 2022). There was a limitation of sample size also in the present study. The body weight loss, viral load, and lung pathological changes in the BA.2 infected placebo group were lesser compared to BA.1.1 placebo group indicating a lower pathogenicity of BA.2 variant in agreement with the earlier studies (Su et al., 2022; Yamasoba et al., 2022).

The evidence from the present in vivo study shows that COVAXIN® booster immunization tends to broaden the protective immune response and reduces disease severity against the Delta and Omicron variant infections.

**Limitations of the study**

We have not assessed the cellular immune response in the present study because of the limitation of availability of reagents specific for the Syrian hamsters. The interval between the second dose and the third booster dose in the present study was short compared to the real life scenario where the booster doses are taken months later when the immune response starts waning.
Figure 8. Histopathological changes in lungs of hamsters after SARS-CoV-2 Omicron variant challenge

The lungs of BA.1.1 infected hamsters of the (A) placebo group showing interstitial pneumonia with thickened alveolar septa and hemorrhages (black arrows) (scale bar = 100\(\mu\)m) and (B) immunized group showing alveolar capillary congestion on 7DPI (scale bar = 100\(\mu\)m). The lungs of BA.2 infected hamsters of the (C) placebo group showing interstitial pneumonia characterized by diffuse alveolar septal thickening, exudates in alveoli and peribronchial (yellow arrow) and perivascular (black arrows) inflammatory cell infiltration (scale bar = 100\(\mu\)m) and (D) immunized group showing alveolar capillary congestion and a foci of peribronchial inflammatory cell infiltration (black arrows) (scale bar = 100\(\mu\)m).

(E) Cumulative histopathological score of the lung lesions after Omicron variant infection in hamsters. The mean and standard deviation are plotted on the graph. The significant differences between the experimental groups (\(n = 4\)/group) were assessed using Mann Whitney test and the p value is plotted above the bars of the respective groups. p values less than 0.05 were considered statistically significant.

(F) Lung section of a naive control hamster showing normal lung histology (scale bar = 100\(\mu\)m).

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AUTHOR CONTRIBUTIONS

P.D.Y. and S.M. conceived and designed the study. K.M. and B.G. prepared and provided the vaccine formulations. S.M. performed the animal experiments. P.D.Y., S.M., A.S.A., G.S., and G.D. performed the laboratory work planning. G.D. and G.S. performed the neutralization assays. A.S.A. and R.J. performed the ELISA. A.K. and K.W. assisted in animal experimentation, data collection, and performed sample collection. H.D. performed the virus neutralization assay. A.K., K.W., J.Y., and P.G. performed the sample processing in the laboratory. P.D.Y. and S.M. drafted the manuscript. K.M., B.G., and P.A. substantively revised the manuscript. All authors reviewed the manuscript and agree to its contents.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Goat anti-hamster IgG horseradish peroxidase | Thermo Fisher Scientific | Cat # 31,115/RRID_AB_228247 |
| Bacterial and virus strains |        |            |
| SARS-CoV-2 Delta variant | ICMR-National Institute of Virology, Pune | EPI_ISL_2400521 |
| SARS-CoV-2 BA.1.1 variant | ICMR-National Institute of Virology, Pune | EPI_ISL_8542938 |
| SARS-CoV-2 BA.2 variant | ICMR-National Institute of Virology, Pune | EPI_ISL_12667336 |
| SARS-CoV-2 B.1 variant | ICMR-National Institute of Virology, Pune | EPI_ISL_420546 |
| Chemicals, peptides, and recombinant proteins |        |            |
| S1-Receptor Binding Domain protein | Labcare, India | Cat # F5001 |
| Five percent skimmed milk | Difco, Thermo Fisher Scientific | Cat #. 232100 |
| Tween-20 | Sigma-Aldrich | Cat # P1379 |
| 3, 3', 5', 5'-tetramethylbenzidine | Sigma Aldrich | Cat # T444 |
| COVAXIN® | Bharat Biotech India Private Limited | N/A |
| Minimum Essential Medium | Thermo Fisher Scientific | Cat # 11534466 |
| Foetal Bovine serum | Sigma-Aldrich | Cat # F4135 |
| Penicillin/Streptomycin | Sigma-Aldrich | Cat # P4333 |
| Critical commercial assays |        |            |
| MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit | Thermo Fisher Scientific | Cat #A42352 |
| Experimental models: Cell lines |        |            |
| ATCC® CCL-81™ | ATCC | Cat#ATCC-CCL-81 |
| Experimental models: Organisms/strains |        |            |
| Golden Syrian hamsters (Mesocricetus auratus) | Indian Council of Medical Research-National Institute of Virology, Pune | N/A |
| Oligonucleotides |        |            |
| E gene primer | Eurofins Genomics India Private Limited | Cat# I-0000637 |
| For: ACAGGTACGTAAATGTAAATACGCT |        |            |
| Rev: ATATTGCAGTCAGTACGCCACACA |        |            |
| Probe: FAM-ACACTACGCTCCTGCTGCTTTCG-BHQ |        |            |
| N gene primer | Eurofins Genomics India Private Limited | Cat# I-0000637 |
| For: 5'-CCAACCAACTTTGGATCTCTTGTA-3' |        |            |
| Rev: 5'-ATGCGGATAGCACTAAATATTAA-3' |        |            |
| Probe: 5'-FAM-ACCCCGCATTACGTTTGGTGGACC-3 BHQ |        |            |
| Software and algorithms |        |            |
| PRISM | GraphPad software | Version 9.4.2 |

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to the lead contact, Dr. Pragya D. Yadav (hellopragya22@gmail.com).

Materials availability
This study did not generate new unique reagents.
Data and code availability

- This paper includes all datasets generated and analysed during this study. This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.
- All the data related to the experiments are available in the manuscript and supplementary data.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study design

The experiments were conducted as per the approval of Institutional Animal Ethics Committee (Approval number: NIV/IAEC/2020/MCL/10) and Institutional Biosafety Committee (Approval number: NIVIBSC/30.05.2020/01) and were performed according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA, 2018). Seventy two, 8–10 week old, female Syrian hamsters (Mesocricetus auratus) procured from a CPCSEA licensed breeding facility was used in the study. Syrian hamsters were housed in the individually ventilated cages with access to ad libitum food and water in the containment facility. The animals were acclimatized to the laboratory conditions for a period of 7 days before the experiments. The animals were observed daily for their activity and a weight loss of 15% was set as humane end point for the study.

Virus

SARS-CoV-2 variants, Delta (EPI_ISL_2400521), BA.1.1 (EPI_ISL_8542938), BA.2 (EPI_ISL_12667336) and B.1 (EPI_ISL_420546) isolated from a patient’s throat/nasal swab sample in Vero CCL-81 cells was used for the study.

COVAXIN® immunization and virus challenge

In the first set of experiments, 40 hamsters were divided into 4 groups of 10 animals each. Animals of two groups (Group 2 and 4) were immunized intramuscularly with 0.2 mL of COVAXIN®. Group 2 received 2 doses of COVAXIN® on day 0 and 14 and group 3 received 3 doses of COVAXIN® on Day 0, 14 and 35. Group 1 and 3 were given 2 and 3 doses of 0.2 mL of phosphate-buffered saline respectively. The animal caretakers and the technical staff were blinded about the group allocation. Blood samples were collected on day 28 from hamsters which received two doses of COVAXIN® and on day 48 from hamsters which received 3 doses of vaccine and were assessed for IgG and neutralizing antibody response. The hamsters of Group 1 and 2 were challenged intranasally with 0.1 mL (10^5 TCID50/0.1 mL) of SARS-CoV-2 Delta variant on day 35 and the group 3 and 4 on day 60. Following the virus challenge the hamsters were monitored for the body weight changes and the viral RNA shed through throat swabs and nasal wash on 1,3,5 and 7 DPI. Five hamsters/group were sacrificed on day 3 and day 7 and lungs and nasal turbinates were collected to understand the viral load. A portion of the lungs sample was immersion fixed in 10% formalin for histopathological investigations.

In the second set of experiments, 32 Syrian hamsters of either sex were used. Sixteen hamsters were immunized with 3 doses of COVAXIN® and the other 16 were given placebo injections of phosphate buffered saline under isoflurane anaesthesia. Eight hamsters from the COVAXIN® immunized group and eight from the placebo group were infected with 0.1 mL of SARS-CoV-2 BA.1.1 variant (1.9 x 10^5 TCID50/mL) intranasally on day 56 and the rest of the hamsters were infected with BA.2 variant (1.8 x 10^5 TCID50/mL) on day 70 intranasally. The hamsters were observed for body weight changes post virus infection. Nasal wash and throat swab samples were collected on 1,3,5 and 7 DPI. Four hamsters each were sacrificed on day 3 and 7 post infection and blood, lungs and nasal turbinates were collected to understand the viral load and immune response. Lungs samples were collected for histopathological investigations. Immunization, blood collection and virus inoculation were performed under isoflurane anaesthesia and the euthanasia with isoflurane overdose.

METHOD DETAILS

Anti-SARS CoV-2 IgG and S1-RBD ELISA

The anti-SARS-CoV-2 IgG and S1-RBD ELISA were performed as described earlier (Mohandas et al., 2022; Shete et al., 2021). For the anti-SARS-CoV-2 IgG ELISA, plates coated with inactivated SARS-CoV-2 antigen...
were used. Hundred µl of diluted hamster serum samples (1: 100) were added to the wells and were incubated at 37°C. After washing, anti-hamster IgG horseradish peroxidase antibodies in the dilution, 1:3000 were added and incubated for 1 h at 37°C. Hundred µl of substrate, 3',3',5,5'-tetramethylbenzidine (TMB) was added to each well and incubated. The colour reactions were terminated after 10 min and the absorbance was measured at 450 nm.

For the RBD ELISA, plates coated with S1-RBD protein was used. Five percent skimmed milk was used for blocking the wells. Following washing, the serum samples were added and were incubated for 60 min. Hundred µl of anti-hamster IgG horseradish peroxidase was added to each well after washing the plate. The plate was incubated at 37°C for 30 min. TMB substrate was used for colour development. The reaction was terminated using 1 N sulphuric acid and the optical density (OD) was measured at 450 nm.

**Plaque reduction neutralization test**

The assay was performed using B.1, Delta, BA.1.1 and BA.2 variants as described earlier (Deshpande et al., 2020). Four-fold serially diluted hamster sera mixed with an equal amount of virus suspension was incubated for 1 h at 37°C. The sera-virus mixture was added on to a 24-well tissue culture plate with Vero CCL-81 cells and was incubated for an hour at 37°C. Following incubation, an overlay medium containing 2% carboxymethyl cellulose was added and the plate was further incubated at 37°C for 4 days. The plates were stained with 1 per cent amido black for an hour and PRNT 50 titres were calculated.

**Quantitative real-time RT-PCR**

Nasal wash and throat swab samples collected in 1mL viral transport medium and weighed organ samples (lungs, nasal turbinate) homogenized in 1 mL sterile tissue culture media were used for RNA extraction. MagMAX™Viral/Pathogen Nucleic Acid Isolation Kit was used for the procedure as per the manufacturer’s instructions. Published primers targeting E gene was used for viral RNA estimation and targeting N gene were used for the subgenomic RNA estimation (Choudhary et al., 2020; Perera et al., 2020).

**Virus titration in vero cells**

The lungs, nasal turbinate and nasal wash samples were used for virus titration in Vero CCL81 cells. Twenty four-well plate with Vero CCL81 monolayers were incubated for 1 h after addition of the hundred microlitre of the sample. The plate was washed after removal of the media and was incubated again with maintenance media containing 2% FBS in a CO2 incubator. The plates were examined for any cytopathic effects daily.

**Histopathology**

The formalin fixed lung tissue samples were processed by routine histopathological techniques for haematoxylin and eosin staining (CULLING, 1974). The lung lesions were scored for six criteria, i.e. for vascular lesions (congestion and hemorrhages), bronchiolar lesions (loss of epithelium, exudation, degeneration), alveolar lesions (consolidation, septal thickening, hyperplasia), edematous and emphysematous changes, inflammatory infiltration in alveolar interstitium and peribronchial and perivascular inflammatory cell infiltration. The sections were scored on a scale of 0 to 4 for all the six criteria and the cumulative score was compared between the groups.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

For analysis of the data, Graphpad Prism version 9.4.3 software was used. Mann-Whitney test was performed for comparison between the experimental groups. The p-values less than 0.05 were considered to be statistically significant.