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Immunogenicity and protective efficacy of BBV152: a whole virion inactivated SARS CoV-2 vaccine in the Syrian hamster model

Sreelekshmy Mohandas
Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021
Pragya D Yadav (hellopragya22@gmail.com)
Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021
Anita Shete
Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021
Priya Abraham
Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021
Krishna Mohan
Bharat Biotech International Limited, Genome Valley, Hyderabad, Telangana, India, Pin-500 078
Gajanan Sapkal
Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021
Chandrashekhar Mote
Department of Veterinary Pathology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Maharashtra, India, Pin-412801
Dimpal Nyayanit
Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021
Nivedita Gupta
Indian Council of Medical Research, V. Ramalingaswami Bhawan, P.O. Box No. 4911, Ansari Nagar, New Delhi, India, Pin-110029
V K Srinivas
Bharat Biotech International Limited, Genome Valley, Hyderabad, Telangana, India, Pin-500 078
Manoj Kadam
Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021
Abhimanyu Kumar
Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021
Rajlaxmi Jain
Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021
Triparna Majumdar
Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021
Gururaj Deshpande

Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021

Savita Patil

Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021

Prasad Sarkale

Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021

Deepak Patil

Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021

Raches Ella

Bharat Biotech International Limited, Genome Valley, Hyderabad, Telangana, India, Pin-500 078

Sai D Prasad

Bharat Biotech International Limited, Genome Valley, Hyderabad, Telangana, India, Pin-500 078

Sharda Sharma

Indian Council of Medical Research-National Institute of Virology, Pune, Maharashtra, India, Pin-411021

Krishna M Ella

Bharat Biotech International Limited, Genome Valley, Hyderabad, Telangana, India, Pin-500 078

Samiran Panda

Indian Council of Medical Research, V. Ramalingaswami Bhawan, P.O. Box No. 4911, Ansari Nagar, New Delhi, India, Pin-110029

Balram Bhargava

Indian Council of Medical Research, V. Ramalingaswami Bhawan, P.O. Box No. 4911, Ansari Nagar, New Delhi, India, Pin-110029

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Abstract

The availability of a safe and effective vaccine would be the eventual measure to deal with SARS-CoV-2 threat. Here, we have developed and assessed the immunogenicity and protective efficacy of an inactivated SARS-CoV-2 vaccine (BBV152) in hamsters. Three dose vaccination regime with three formulations of BBV152 induced significant titres of SARS-CoV-2 specific IgG and neutralizing antibodies. The formulation with imidazoquinoline adsorbed on alum adjuvant remarkably generated a quick and robust immune response. Th_1 biased immune response was demonstrated by the detection of IgG2 antibodies. Post-SARS-CoV-2 infection, vaccinated hamsters did not show any histopathological changes in the lungs. The protection of the hamsters was evident by the rapid clearance of the virus from lower respiratory tract, reduced virus load in upper respiratory tract, absence of lung pathology and robust humoral immune response. These findings confirm the immunogenic potential of BBV152 and further protection of hamsters challenged with SARS-CoV-2.

Highlights

- BBV152, an inactivated vaccine induced potent humoral immune response in hamsters
- Th1 biased immune response was elicited by BBV152
- BBV152 protected Syrian hamsters from SARS-CoV–2 pneumonia

Introduction

Since the first report in December 2019, severe acute respiratory syndrome coronavirus–2 (SARS-CoV–2) has spread at an alarming rate and has infected more than 24 million people until August 31st 2020 (World Health Organization, 2020). Globally, scientific communities are actively engaged and trying to develop suitable vaccine candidates and specific antiviral therapies against this virus. Vaccination is the most significant pharmaceutical intervention for the prevention of any infectious disease impacting the health of communities worldwide. Accelerated efforts are being taken for the development of a safe and effective vaccine worldwide which is evident by the number of vaccine candidates under preclinical and clinical evaluation. According to the World Health Organization draft landscape document on COVID–19 candidate vaccines published on 23rd August 2020, 33 and 143 vaccine candidates are under clinical and preclinical studies respectively (World Health Organization, 2020).

Currently along with the conventional vaccine development platforms, advanced technologies are being used for the development of vaccines against SARS-CoV–2 like messenger RNA, DNA, viral vectors, recombinant subunit proteins, virus-like particles etc (Pandey et al., 2020). Despite the advances in vaccine design technologies, the development of an inactivated vaccine remains the most simple and relatively less expensive approach to produce a safe and effective vaccine. Inactivated vaccines have been used effectively to curb many infectious diseases in the past (Sanders et al., 2015). Recently, two inactivated SARS-CoV–2 vaccine candidates have shown promising results in preclinical trials (Gao et al., 2020, Wang et al., 2020). Among various small laboratory animal models, Syrian hamsters was found to

be a suitable model for SARS-CoV–2 research, as the virus has been shown to replicate both in the upper and lower respiratory tract (Mohandas et al., 2020). The pulmonary pathology and lung viral load coincides with weight loss in hamsters during the first week of infection (Chan et al., 2020), and can be used for the assessment of protective efficacy of vaccine candidates. This animal model has been successfully used to evaluate medical countermeasures for SARS-CoV–2 by multiple research groups (Luan et al., 2020, Chan et al., 2020, Imai et al., 2020).

We have developed a whole virion inactivated vaccine candidate (BBV152) using β-propiolactone (BPL) inactivation method. The vaccine candidate along with aluminum hydroxide adjuvant alone or with aluminum hydroxide chemisorbed with imidazoquinoline was found to be immunogenic and safe in the preclinical studies on laboratory mice, rats and rabbits. Here, we report the immunization of Syrian hamsters with the vaccine candidate (BBV152) and evaluation of protective efficacy against SARS-CoV–2 by carrying out virus challenge experiment.

Results

Optimization of SARS-CoV–2 challenge dose in Syrian hamsters

For assessment of the SARS-CoV–2 challenge dose after immunization a 10 fold serial dilutions of $10^{5.5}$ Median Tissue Culture Infectious Dose (TCID50) were used. One hundred microliter from each of the 5 dilutions ($10^{5.5}$, $10^{4.4}$,$10^{3.5}$, $10^{2.5}$, and $10^{1.5}$) was inoculated intranasally in 5 groups of 6 hamsters each. On 3 day post infection (DPI), 3 hamsters each from all the 5 groups were sacrificed and the lung viral titres were measured. Virus load was found to be similar in all the hamsters by real time RT-PCR irrespective of the virus dilutions inoculated. Further to confirm this finding virus titration was performed on the same lung samples and TCID50 titre ranging between $10^{5.48}$ to $10^{5.58}$ was observed in all samples on 3DPI (Figure S1). Similarly on day 14, the remaining hamsters were sacrificed and lung samples were found to show a titre of $10^{2.5}$ TCID 50/ ml in all the animals, indicating that viral inoculum could induce disease in all hamsters (Figure S1). On histopathological analysis, lungs samples of the hamsters inoculated with the $10^{5.5}$TCID50 and $10^{4.5}$ TCID50 dilutions collected on 3DPI showed mild inflammatory changes indicating beginning of pneumonia, whereas other groups showed minimal or no changes. On 14 DPI, the lung pathological changes were found minimal in all groups indicating recovery from infection. Even though the lung viral titres in all groups were similar irrespective of the dose of virus inoculums, the lung pathological changes indicated the rapid induction of pneumonic changes in $10^{5.5}$ and $10^{4.5}$ dilutions. Hence, a virus dilution dose of $10^{5.5}$ TCID50 was used for virus challenge.

Clinical observations during BBV152 immunization period and post SARS- CoV–2 challenge

We immunized four groups of 6–8 week old Syrian hamsters (9 hamsters in each group), with phosphate buffered saline (group I), 6µg of BBV152 with Algel1 (group II), 3µg of BBV152 with Algel2 (group III) and 6µg of BBV152 with Algel2 (group IV) (Figure 1). All the hamsters received two booster doses on day 14 and day 35. Rectal temperature remained within the normal range and no clinical signs were observed in all the groups throughout the immunization period. The body weight increased until 7 weeks post-immunization (Figure 2A), but following SARS-CoV–2 infection on day 50, decrease in body weight was observed in all the groups (Figure 2B). However, the percentage decrease in vaccinated groups were lesser compared to the group I. The decrease in body weight of group III hamsters was less as compared to other groups (Figure 2).

Inactivated whole virion vaccine candidates induced specific IgG /neutralizing antibody and Th_1 biased immune response

Anti-SARS-CoV–2 IgG antibody response was detected by 3 weeks in 8/9 hamsters of group IV with an average OD of 0.62, 8/9 hamsters in group III with an average OD of 0.42 and in 2/9 hamsters (average OD = 0.285) of group II. On day 48, IgG antibody response was found to be increasing in the vaccinated groups with an average OD of 1.32 in group IV (9/9 hamsters), 1.2 in group III (9/9 hamsters), 0.55 in group II (9/9 hamsters) (Figure 3A, C). All the animals in group I remained negative for IgG antibody during immunization period whereas post virus challenge, 2/3 hamsters showed IgG positivity by 7 DPI and 3/3 by 14 DPI (average OD = 0.29) in the group I (Figure 3B). In the vaccinated groups, an increasing trend with an average OD of 0.84, 0.97 and 0.91 was observed on days 3, 7 and 15 DPI respectively (Figure 3D). No significant difference was observed in the IgG antibody response post-infection in group III and IV (Figure 3A -E).

IgG antibody response in vaccinated hamsters was further characterized to determine the IgG subclass profiles. On sub-typing IgG2 was detected in all the IgG antibody positive samples whereas it was negative for IgG1 during immunization and post-infection phase (Figure 3E). All three formulations of vaccine candidates significantly induced IgG2 with an increasing trend post-infection indicating a Th_1 biased immune response (Figure 3 A-E).

Neutralizing antibody (NAb) started appearing in the immunized groups at 3rd week of immunization and increased till 7^{th} week with highest titre (mean = 28810) in group III (Figure 3F). After virus infection the highest titre of NAb (mean = 85623) was seen in group III animals on 15 DPI. Group I did not show NAb response during immunization phase and after virus infection till 15 DPI (Figure 3G).

Detection of SARS-CoV–2 genomic RNA (gRNA) in swabs/ organ samples post virus challenge

SARS-CoV–2 viral genome copy number in throat swabs (TS) of group I were significantly high compared to other groups on 3, 5 and 7 DPI (Figure 4A). The viral gRNA in group I persisted till 10 DPI, whereas it was cleared in all the vaccinated groups by 5 DPI. Higher copy numbers of viral gRNA were detected in the nasal washings of group I (Figure 4B).

Lungs (Figure 5A), Nasal turbinates (Figure 5B) and trachea (Figure 5C) of group I showed higher viral gRNA copy number compared to other groups on 3 and 7 DPI. Trachea was cleared of viral gRNA by 7 DPI in all the groups. Complete gRNA clearance was observed from lungs of group III and IV on 7 DPI and from group II by 15 DPI. Nasal turbinates viral gRNA persisted in all the groups till 15 DPI, but with lower copy numbers in vaccinated groups compared to group I. No viral subgenomic (sg) RNA was detected in TS, nasal wash, nasal turbinate or trachea of animals of vaccinated groups. However viral sgRNA was detected in lungs (3/3), trachea (1/3) and nasal turbinate (1/3) in group I animals on 3 DPI. The spleen, kidney and small intestine of hamsters of group I showed viral gRNA positivity on 3 DPI (Figure 5D).

Virus titration

Lungs, nasal turbinate and TS samples of group I showed an average titre of 10^6, $10^{5.5}$ and 10^4 TCID50/ml respectively on 3 DPI. In the vaccinated groups III and IV, nasal turbinates showed an average titre of 10^5 and 10^4 TCID50/ml on 3 DPI, whereas the TS and lungs titre was found considerably less ($10^{2.5}$ TCID50/ml) compared to group I. In contrast group II did not show live virus titre in any of the specimens on 3, 7 and 15 DPI. On 7 DPI only nasal turbinates of group I showed virus titre whereas vaccinated groups were negative. This correlates with the decreasing trend of gRNA and sgRNA in immunized groups after 3 DPI.

Pathological and Immuno-histochemistry findings in lungs post virus inoculation

The lungs of the vaccinated groups appeared normal on 3, 7 and 15 DPI (Figure 6B, 6C and 6D) on gross pathology whereas on 7 DPI the lungs of group I showed diffuse areas of consolidation and congestion (Figure 6A). On histopathological examination, lung sections from group I animals showed congestion, haemorrhages, exudations in the alveoli, mononuclear cell infiltration in the alveolar interstitium and pneumocyte hyperplasia on 3 and 7 DPI (Figure 6E, 6F). Occasionally, loss of bronchiolar epithelium was also observed. By 15 DPI, fibro-elastic proliferation with collagen deposition at alveolar epithelial lining were observed in the lungs of group I (Figure 6G). Vaccinated group animals did not show any histopathologic evidence of pneumonia except few congestive foci on 3 DPI (Figure 6H, 6I and 6J). The viral antigen could be detected in alveolar type - II pneumocytes and macrophages on 3, 7 and 15 DPI in the lungs of group I animals (Figure 7A, 7B and 7C) whereas only focal positivity was detected in the vaccinated groups on 3 DPI. On 7 and 15 DPI viral antigen was not detected in the lung sections of vaccinated hamsters from all the groups on immunohistochemistry (IHC) (Figure 7D, 7E and 7F).

Cytokine profile after virus infection

After challenge with the virus vaccinated groups did not have any significant elevation of cytokines i.e TNF-α, IL–4, IL–10, IL–6, IFN-γ and IL–12 whereas in control group I increased level of IL–12 was detected on 3 DPI which further reduced on 7 and 15 DPI (Figure S2).

Discussion

Considering the pandemic scenario of COVID–19 worldwide a safe and effective vaccine is the most appropriate and effective method for control of the rapid spread of this disease. Globally several research groups are working on the development of vaccine candidates for COVID–19. Some of them have progressed to the clinical trials (Doremalen et al., 2020; Mercado et al., 2020; Corbett et al., 2020; Patel et al., 2020; Gao et al., 2020; Wang et al., 2020). Preclinical research in animal models is an important step in evaluating the immunogenicity and protective efficacy of vaccine candidates. It aids in assessing the host response and findings on histopathology helps in evaluating the safety of the vaccine candidates before progressing to clinical trials. Multiple animal models have been used to evaluate the efficacy of SARS-CoV–2 vaccine candidates. Syrian hamster (*Mesocricetus auratus)* is one such model which has been used in diverse research studies on SARS-CoV–2 and seems to be the appropriate model as it mimics the human disease in comparison to other animals (Mohandas et al., 2020, Luan et al., 2020, Chan et al., 2020, Imai et al., 2020; Wang et al., 2019). Here we report the immunogenicity and protective efficacy of the three-dose vaccination regimen of inactivated SARS-CoV–2 vaccine candidate in the hamster model.

SARS-CoV–2 specific IgG titres were observed from 3rd week post-immunization in 90% of animals of Group III and IV, indicating the faster and higher immunogenic potential of BBV152 with imidazoquinoline. By 7 weeks all the vaccinated groups showed seroconversion however an increased titre was observed in group III and IV compared to group II. Detection of IgG2 antibodies in the immunized groups indicates Th_1 biased immune response. Neutralizing antibodies was elevated in all the vaccinated groups post SARS-CoV–2 challenge. Similarly Roberts *et al.* also reported a significant increase in the antibody titres following the two-dose vaccination regimen of inactivated whole virus SARS-CoV vaccine in hamsters (Roberts et al., 2010). Compared to the placebo group, rapid virus clearance was observed in the vaccinated groups from the upper and lower respiratory tract except for nasal turbinates. Viral gRNA in the nasal turbinates persisted till day 15 in all groups. However gRNA copy number was lower in vaccinated groups compared to placebo. Gross and histopathological examination of the lungs of the placebo group had evidence of interstitial pneumonia in the placebo group on 3 and 7 DPI. However vaccinated groups had no evidence of gross and histopathological changes indicating the protective efficacy of BBV152. Lower viral gRNA copy number at 3 DPI, absence of lung pathology and high titers of neutralizing antibodies post-infection demonstrate the protective efficacy of BBV152 in immunised hamsters.

Star Methods

Key Resource table

Reagent/Resource	Source	Identifier
Experimental models: Cell lines		
Vero CCL-81	ATCC	Cat#ATCC-CCL-81
Experimental models: Organisms/strains		
Hamsters: Syrian hamsters	Indian Council of Medical Research-National Institute of Nutrition, Hyderabad, India	--
Virus strain		
NIV-2020-770	ICMR-National Institute of Virology, Pune	hCoV-19/India/770/2020|EPI_ISL_420545| hCoV19/India/2020770/2020|EPI_ISL_420546|
Antibodies		
Biotin mouse anti-Armenian and Syrian Hamster IgG1	BD Biosciences	Cat#554007
Biotin mouse anti-Armenian and Syrian Hamster IgG2	BD Biosciences	Cat#554025
Anti-mice HRP	Dako	Cat #P044701-2
Chemicals		
Imidazoquinoline	Viro Vax	
Alum	Brenntag	
b-Propionolactone	Ferak	
Minimum Essential Medium (MEM)	Thermo Fisher Scientific	Cat # 11534466
Dulbecco's Modified Eagle Medium	Sigma-Aldrich	Cat # D5796
Foetal Bovine serum	Sigma-Aldrich	Cat # F4135
Penicillin/Streptomycin	Sigma-Aldrich	Cat # P4333
Skimmed milk powder	Difco	Cat #232100
3,3',5,5'-Tetramethylbenzidine	Clinical science product	Cat#01016-1-1000

(TMB) substrate		
Critical Commercial Assays		
Hamster Interleukin ELISA Kit	Immunotag	Cat #ITE150025, ITE150027,ITE150010, ITE150004,ITE150058,ITE150028,ITE150030
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	Thermo Fisher Scientific	Cat #A42352
Software		
PRISM	GraphPad software	Version 8

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 Statement

The published article includes all datasets generated or analysed during this study.

Experiment model and subject details

Cells and virus

Vero CCL81 cells were grown in Minimum Essential Media (MEM) (Thermo fisher Scientific, USA) supplemented with 2% foetal bovine serum (FBS) (Sigma-Aldrich, USA). SARS-CoV-2 strain, NIV-2020-770 isolated from a patient's throat/ nasal swab sample at Indian Council of Medical Research- National Institute of Virology, Pune was passaged up to three times in Vero CCL81 cells before use (Sarkale et al., 2020). The infectious virus titre was found to be $10^{6.5}$ tissue culture infective dose 50 (TCID50)/ml.

Ethics statement

The study was approved by the Institutional Project Review Committee, Institutional Animal Ethics Committee and Institutional Biosafety Committee of Indian Council of Medical Research-National Institute of Virology, Pune. All the experiments were performed as per the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA, 2003).

Immunization of hamsters

Thirty-six, 6- 8 week old, female Syrian hamsters were divided into four groups, viz., Group I, II, III, and IV of 9 hamsters each. The hamsters were housed in individually ventilated cages with *ad libitum* food and water. Group I was administered with phosphate-buffered saline (PBS), group II with 6µg of vaccine candidate along with Algel 1, group III with 3µg of vaccine candidate with Algel 2, and group 4 with 3µg of vaccine candidate with Algel 2. Animals of each group were immunized with 0.1 ml of PBS/vaccine formulations intramuscularly in the left hind leg under isoflurane anaesthesia 0, 14, and 35 days. Post immunization hamsters were observed daily for clinical signs and injection site reaction. Rectal temperature was monitored every 24 hours for 3 days post-immunization and weekly thereafter. Body weight was measured every alternate day for the first week and weekly thereafter. The hamsters were bled on day 12, 21, and 48 post-immunization to check for antibody response.

Dose optimization study in hamsters

For optimization, of the intranasal virus challenge dose, SARS-CoV-2 dilutions ranging from $10^{1.5}$ $TCID_{50}$/0.1ml to $10^{5.5}$ $TCID_{50}$/ml were inoculated in 5 groups of 6 Syrian hamsters each in containment facility. Three hamsters from each group were sacrificed on days 3 and 14 post-inoculation to check for viral load in the lungs by real-time RT-PCR/virus titration and lung pathology.

Challenge study in hamsters

The immunized hamsters were challenged with 0.1 ml of $10^{5.5}$ TCID50 SARS-CoV-2 virus intranasally on the eighth-week post-immunization (day 50) in the containment facility of ICMR-National Institute of Virology, Pune under isoflurane anaesthesia. Throat swabs were collected in 1 ml virus transport media on every alternate day post-inoculation for viral load estimation. Three hamsters from each group were euthanized on 3, 7 and 15 DPI to collect throat swab, nasal wash, rectal swab, blood and organ samples for viral RNA estimation, titration, histopathology, and immunological analysis.

Method details

Inactivated SARS-CoV-2 whole virion vaccine (BBV152)

SARS-CoV-2 strain (NIV-2020-770) isolated at ICMR-NIV, Pune was propagated in Vero CCL81 cells and harvested on observation of cytopathic effect in the cells. At BBIL BPL (Ferak, Germany) was added to the virus harvest following filtration and stabilization of the harvest using a buffer. The mixture was kept at 2-8˚C with continuous stirring for 24 hrs and was further hydrolysed by incubating at 37˚C for 2hrs. Column chromatography was used for further purification and the process intermediate was concentrated to prepare the whole virion vaccine. Two different antigen concentrations (3 µg and 6 µg) and 2 adjuvants namely Algel 1 (Alum) and Algel 2 (TLR 7/8 (imidazoquinoline) agonist adsorbed alum) in combinations were used for the study. The vaccine formulations evaluated in the study were 6 µg antigen with Algel1, 3 µg with Algel 2, and 6 µg with Algel 2.

Enzyme-linked Immunosorbent Assay

Ninety-six well microtitre plates were coated with 1:10 diluted inactivated SARS-CoV-2 antigen with carbonate buffer (pH 9.5) overnight at 4 °C. Subsequently, wells were blocked with liquid plate sealer (CANDOR Bioscience GmbH, Germany) for two hours at room temperature (25-30°C). The wells were washed 5 times with phosphate-buffered saline with 0.05% Tween 20 (PBS-T) and were incubated at 37°C for one hour with 100µl of diluted hamster serum samples (1: 100). Negative control was added to each plate. After 5 washes with PBS-T, anti-hamster IgG antibodies 1:3000 (Thermoscientific, USA) were added and incubated for 1 hour at 37°C. Following 5 washes with PBS-T, 100 µl of substrate, 3’,3’5,5’-tetramethylbenzidine (TMB) was added to each well. The colour reactions were developed for 10 minutes and after termination, absorbance was measured at 450 nm. Serum IgG titres were determined by testing serial 10-fold dilutions of each sample, starting from 1:100 dilution. Titre values were determined as the highest dilution at which the optical density was more than 0.2 and positive/negative (P/N) ratio above 1.5.

For antibody sub-typing, plates were coated with antigen and blocked as described earlier. Hamster serum, 1:100 diluted in 1% bovine serum albumin in 1× PBST was added to each well and incubated for 1 hour at 37°C. After washing the plates 5 times with 1× PBST wells were probed with biotinylated anti-Syrian hamster IgG1 /IgG2 antibodies diluted at 1:10000 (BD biosciences, USA) and incubated for 1 hour at 37°C. After washing, the plates were incubated with Streptavidin-horseradish peroxidase 1:8000 (Thermo-scientific, USA) for 30 minutes at 37°C. The reaction was read as described earlier.

Plaque Reduction Neutralization test

The assay was performed as described by Gururaj et al., 2020. Briefly, a four-fold serial dilution of hamster serum samples was mixed with an equal amount of virus suspension and incubated at 37°C for 1 hour. Further 0.1 ml of the mixture was inoculated in a 24-well tissue culture plate containing a confluent monolayer of Vero CCL-81 cells. The plate was incubated at 37°C for 60 min and overlay medium (2% carboxymethyl cellulose with 2% FBS in 2X MEM) was added to the cell monolayer, which

was further incubated at 37°C in 5% CO2 incubator for 4-5 days and $PRNT_{50}$ titres were calculated as described earlier.

Cytokine analysis

The serum cytokine levels (TNF-α, IFN-γ, IL-4, IL-6, IL-10 and IL-12) were assessed in hamsters post challenge at 3, 7, and 15 days. An ELISA based commercial assay (Immunotag, USA) was used for the hamster specific cytokine quantitation. For this, plates pre-coated with hamster specific cytokine antibody were used and a streptavidin based HRP system was used for detection and the absorbance was measured at 450 nm.

Real-time RT-PCR for detection of genomic and subgenomic viral RNA

Nasal wash, throat swab and rectal swab samples were collected in 1ml viral transport medium and weighed organ samples (lungs, nasal turbinate, trachea, spleen, kidney and intestine) were triturated in 1 ml sterile tissue culture media, using a tissue homogenizer (Eppendorf, Germany) and 200 µl of the homogenate/ swab specimens were used for further RNA extraction using MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit as per the manufacturer's instructions. Real-time RT-PCR was performed for E and RdRp2 gene for SARS-CoV-2 as well as for detection of sgRNA of E gene using published primers (Choudhary et al., 2020 and Wolfelet al., 2020)

Virus titration

The lungs, nasal turbinate and throat swab samples from 3, 7, and 15 DPI were used for virus titration in Vero CCL81 cells. 100 ml of the sample was added onto a 24-well plate with Vero CCL81 monolayers and incubated for one hour at 37°C. The media was removed and the cell monolayer was washed with PBS. The plate was incubated with maintenance media with 2% FBS in a CO2 incubator. The plate was examined daily for any cytopathic effects (CPE) using an inverted microscope (Nikon, Eclipse Ti, Japan). The culture supernatant from the wells showing CPE was confirmed by real-time RT-PCR (Gururaj et al., 2020).

Histopathology and immunohistochemistry

Lungs samples collected during necropsy were fixed in 10% neutral buffered formalin. The tissues were processed by routine histopathological techniques for hematoxylin and eosin staining. Duplicate sections were taken for immunohistochemical evaluation. An in-house developed anti-SARS-CoV-2 mouse polyclonal serum was used as the primary antibody for detection. The tissue sections were rehydrated

and antigen retrieval was performed using 0.3% hydrogen peroxide in methanol. The slides were incubated with 1: 500 dilution of primary antibody for an hour and an anti-mouse HRP antibody (Dako, USA) was used as a secondary antibody. For detection, 3, 3’-diaminobenzidine tetrahydrochloride substrate, and hydrogen peroxide were used.

Data analysis

For analysis of the data, Graphpad Prism version 8.4.3 software was used. The statistical significance was assessed using the Kruskal-Wallis test Dunn's multiple comparisons test. Two-tailed Mann-Whitney test was performed between the control and the vaccinated groups if the p-value for the Kruskal-Wallis test was found to be significant; p-values less than 0.05 were considered to be statistically significant.

Declarations

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Author’s Contribution

SM, PDY, KM, PA, NG and BB conceived and designed the study. KME, RE, VKS and SDP performed vaccine design and production. PDY, SM performed the planning of animal experiments. SM, PDY, MK, AK, CM performed the animal experimentation. PDY, AS, GS, GD, DN, DYP performed the laboratory work planning and data analysis. GD, SP, RJ, TM, SS, PS performed sample processing in the laboratory. PDY, SM, DN, AS and DYP have drafted the manuscript. PDY, SM, AS, DYP, KM, PA, NG, SP and BB substantively revised it. All authors reviewed the manuscript and agree to its contents.

Declaration of interests

The authors declare no competing interests.

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 Statement

The published article includes all datasets generated or analysed during this study.

Ethics statement

The study was approved by the Institutional Project Review Committee, Institutional Animal Ethics Committee and Institutional Biosafety Committee of Indian Council of Medical Research-National Institute of Virology, Pune. All the experiments were performed as per the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA, 2003).

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Figures

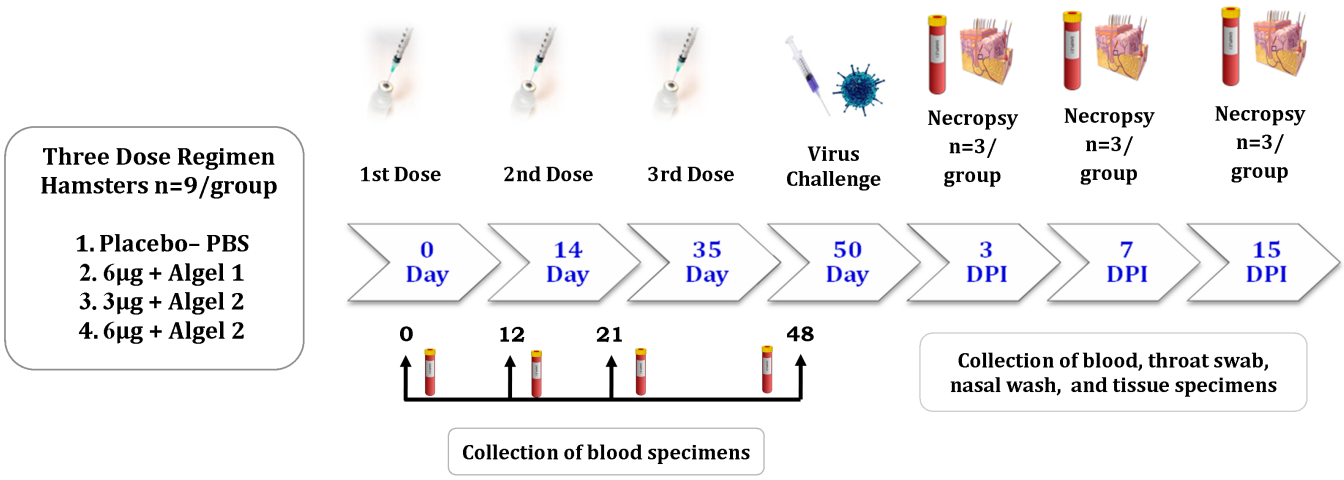

Figure 1

Schematic presentation of experiments in hamsters: Three doses of placebo were administered to the first group of the animals which were controls in the study. Three different inactivated SARS-CoV-2 vaccine formulations (3 doses) were administered to the three groups of animals. All the animals were challenged at 15 days after the third dose. Samples were collected at different time points of immunization period and post-infection as indicated in the figure. Necropsy was performed for three hamsters from each group at 3, 7, and 15 DPI.

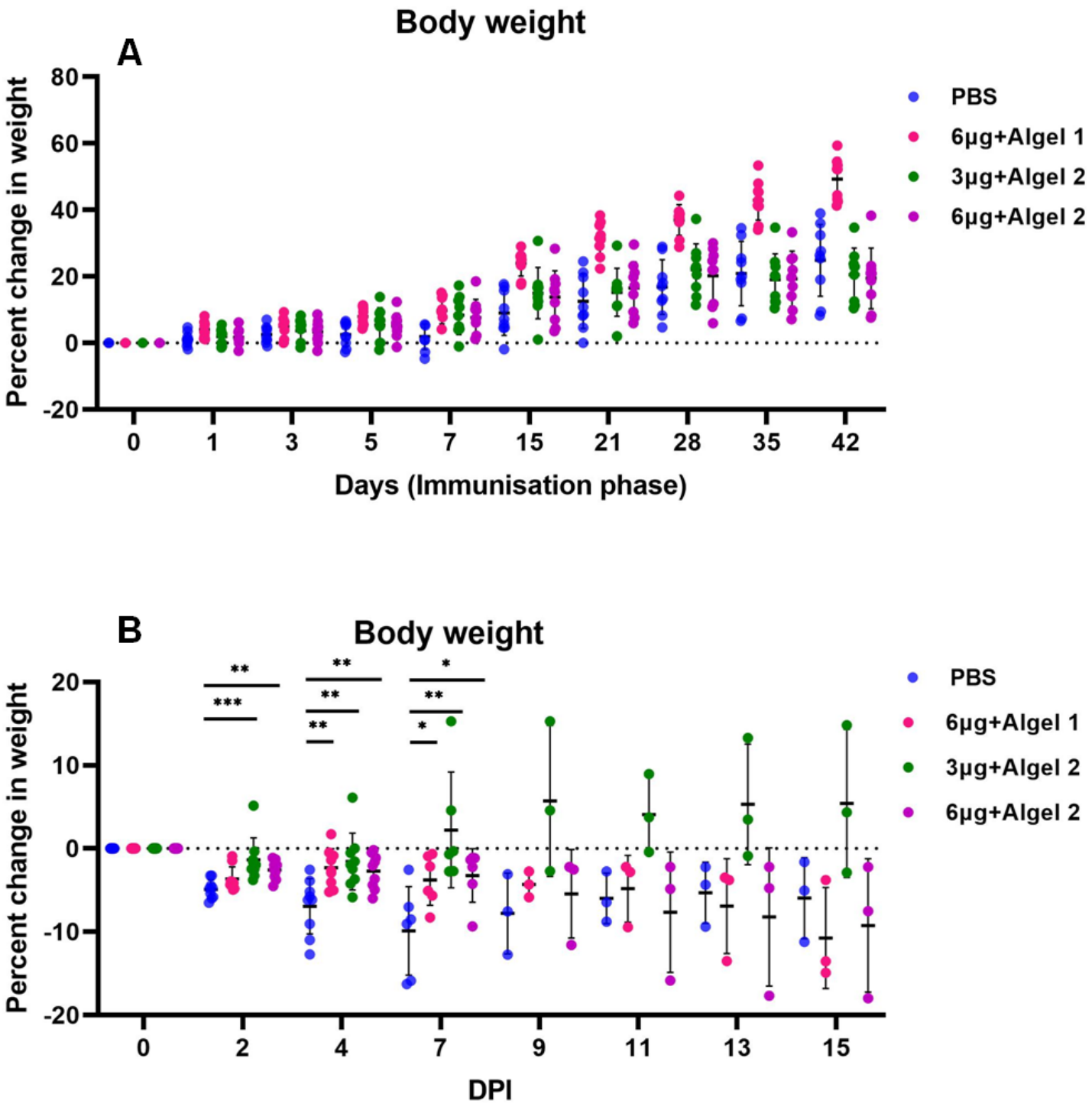

Figure 2

Percent body weight gain/loss in hamsters. A. Percentage of body weight gain in hamsters during the immunization period. B. Percent difference in body weight in hamsters post SARS-CoV-2 challenge. Mean along with standard deviation (SD) is depicted in the scatter plot. The statistical significance was assessed using the Kruskal-Wallis test followed by the two-tailed Mann-Whitney test between the two groups; p-values less than 0.05 were considered to be statistically significant.

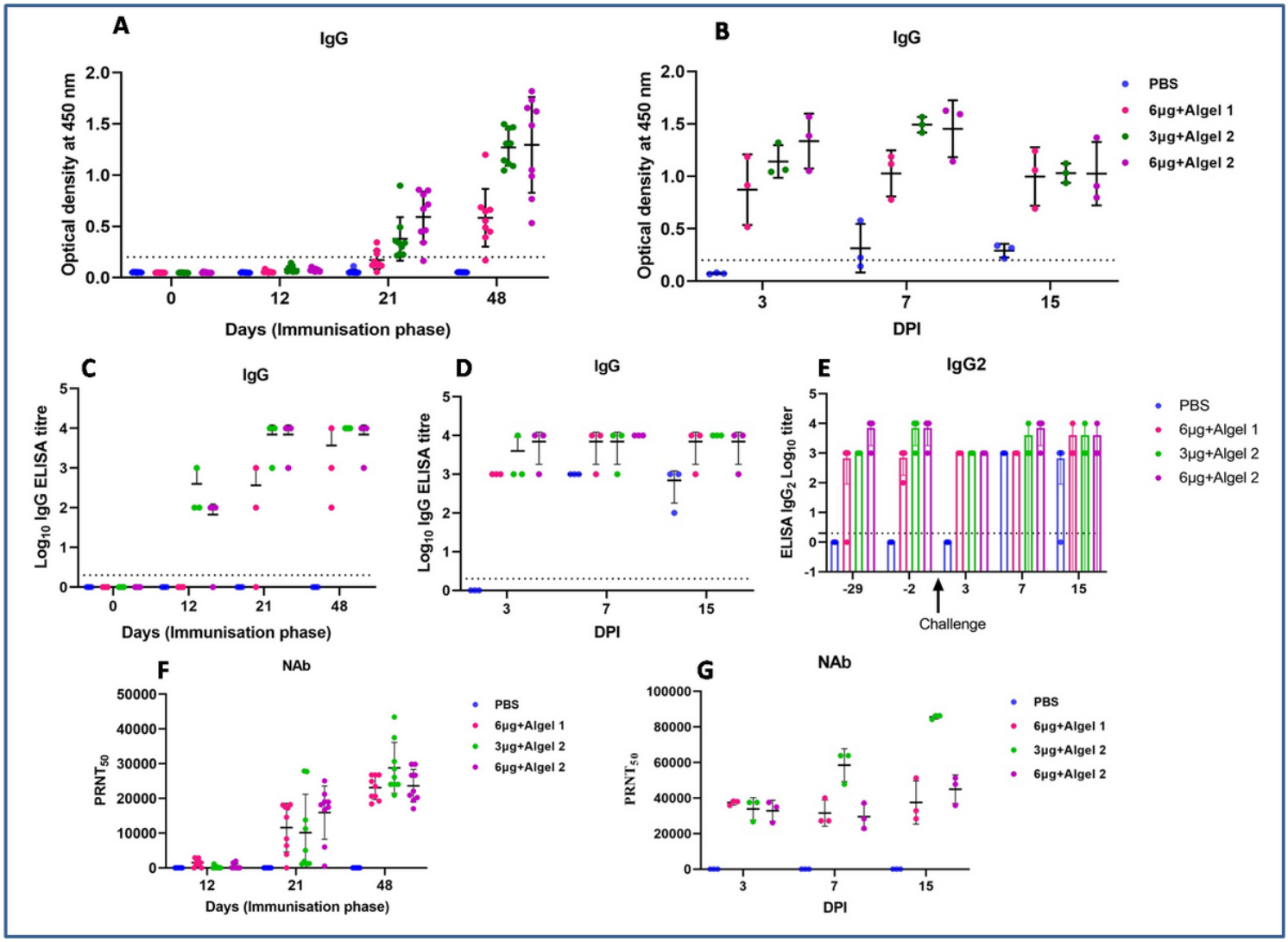

Figure 3

Humoral response in vaccinated animals: (A) IgG antibody response for all groups of animals observed on 12, 21 and 48 days. (B) IgG antibody response at post-infection (3, 7 and 15 DPI) for all groups of animals. (C) Comparison of IgG antibody titres between groups on 12, 21 and 48 days. (D) Comparison of IgG antibody titres between groups post virus challenge at 3, 7 and 15 DPI. (E). Comparison of IgG2 antibody titres between groups during immunization period at 21 and 48 days and post virus challenge at 3,7,15 DPI. (F). Comparison of NAb titres response during a three-dose vaccine regime for all groups of animals observed on 12, 21 and 48 days (G). Comparison of NAb titres response post virus challenge on 3, 7 and 15 DPI. Mean along with standard deviation (SD) is depicted in the scatter plot. The statistical significance was assessed using the Kruskal-Wallis test followed by the two-tailed Mann-Whitney test between the two groups; p-values less than 0.05 were considered to be statistically significant. The dotted lines indicate the limit of detection of the assay.

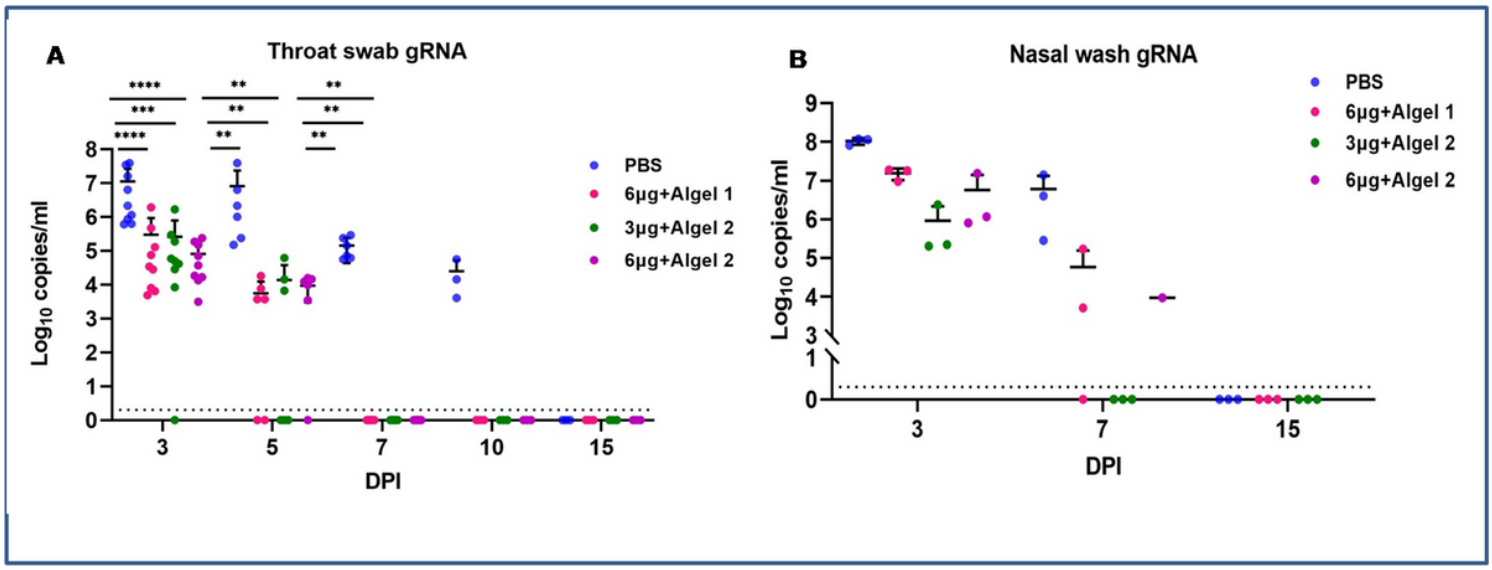

Figure 4

Log10 plot for the genomic viral RNA detection in throat swab and nasal wash after virus challenge. Genomic viral RNA load in (A) Throat Swab collected at 3, 5, 7, 10, and 15 DPI for the all groups (B) Nasal wash collected at 3, 7, and 15 DPI for the all groups. Mean along with standard deviation (SD) is depicted in the scatter plot. The statistical significance was assessed using the Kruskal-Wallis test followed by the two-tailed Mann-Whitney test between the two groups; p-values less than 0.05 were considered to be statistically significant. The dotted lines indicate the limit of detection of the assay.

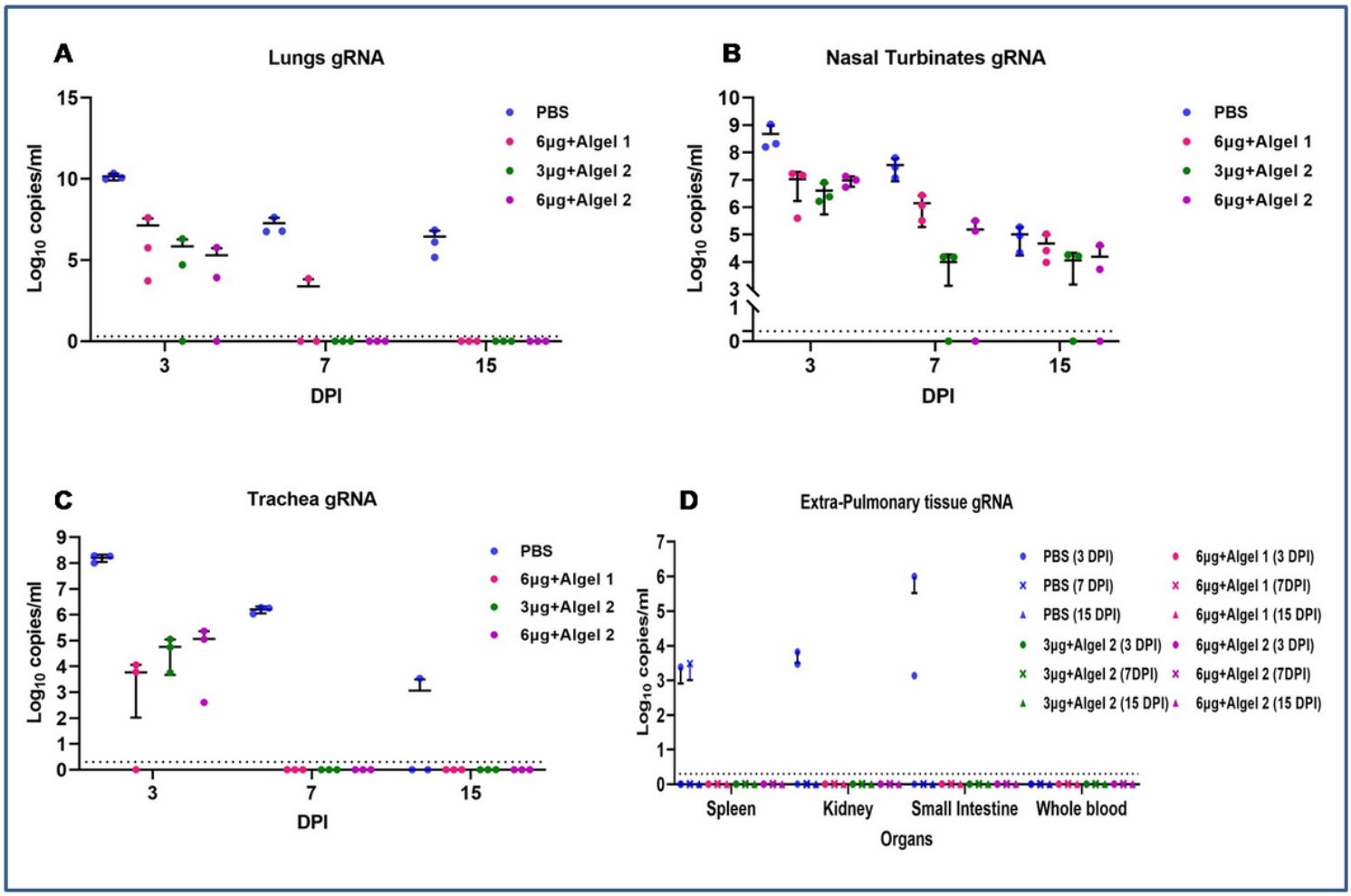

Figure 5

Log10 plot of the genomic viral RNA detection in the respiratory tract and extra pulmonary specimens. Genomic viral RNA load in (A) Lung, (B) Nasal turbinates (C) Trachea (D) Extra pulmonary organs at 3, 7, and 15 DPI. Mean along with standard deviation (SD) is depicted in the scatter plot. The statistical significance was assessed using the Kruskal-Wallis test followed by the two-tailed Mann-Whitney test between the two groups; p-values less than 0.05 were considered to be statistically significant. The dotted lines indicate the limit of detection of the assay.

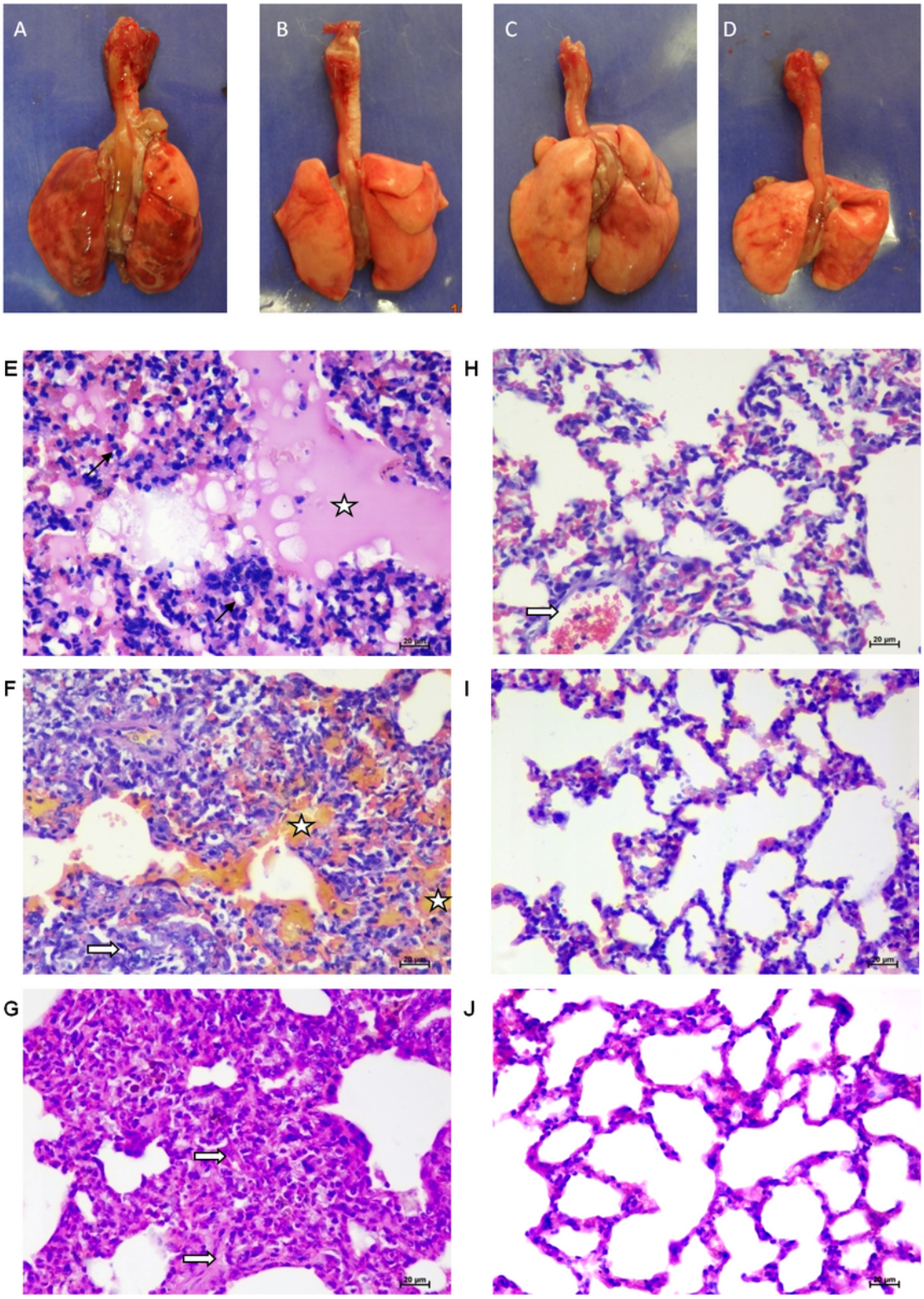

Figure 6

Gross and histopathological observations of lungs in hamsters post virus inoculation. A) Lungs of hamster from group I on 7 DPI showing diffuse areas of consolidation and congestion in the left and right lower lobe with few congestive foci in right upper lobe. Lungs from B) group II, C) group III and D) group IV showing normal gross appearance on 7 DPI. E) Lung tissue from group I on 3 DPI showing acute inflammatory response with diffuse alveolar damage, hemorrhages, inflammatory cell infiltration (black

arrow), hyaline membrane formation (white arrow) and accumulation of eosinophilic edematous exudate (star). F) Lung tissue of group I on 7 DPI showing acute interstitial pneumonia with marked alveolar damage, thickening of alveolar and accumulation of mononuclear cells and macrophages (white arrow), and lysed erythrocytes in the alveolar luminal space (star). G) Lung tissue from group I on 15 DPI depicting interstitial pneumonia with marked thickening of alveolar septa with type-II pneumocyte hyperplasia and fibro-elastic proliferation with collagen deposition at alveolar epithelial lining (white arrow). Lung section from group II showing no evidence of disease H) on 3 DPI few congestive foci I) on 7 DPI J) on 15 DPI.

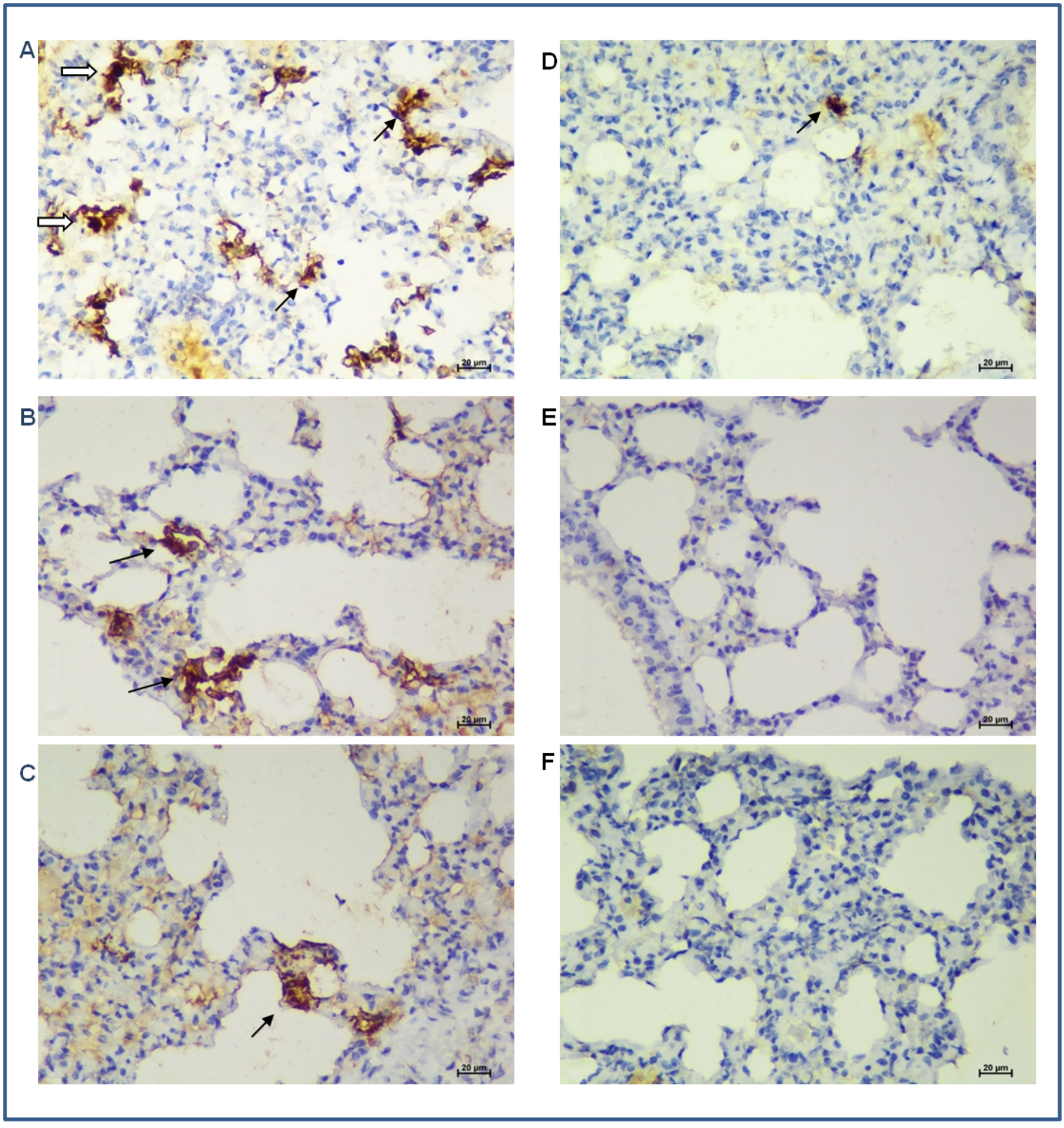

Figure 7

Immunohistochemistry findings in lungs of hamsters post virus challenge. Left panel depicts group treated with placebo and the right panel shows vaccinated group II animals. Lung section from group I showing viral antigen A) on 3 DPI in alveolar type-II pneumocytes (black arrow) and in alveolar macrophages (white arrow) B) on 7 DPI in alveolar type-II pneumocytes (black arrow) C) on 15 DPI in type-II alveolar pneumocytes (black arrow). Lung section from group II showing viral antigen D) on 3 DPI

in alveolar macrophages (black arrow) E) on 7 DPI in alveolar epithelium and alveolar macrophages F) on 15 DPI in the alveolar epithelium and alveolar macrophages.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigureS1.png
- SupplementaryFigureS2.png