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Short communication

Protective efficacy of a plant-produced beta variant rSARS-CoV-2 VLP vaccine in golden Syrian hamsters

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ABSTRACT

In the quest for heightened protection against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants, we engineered a prototype vaccine utilizing the plant expression system of *Nicotiana benthamiana*, to produce a recombinant SARS-CoV-2 virus-like particle (VLP) vaccine presenting the S-protein from the Beta (B.1.351) variant of concern (VOC). This innovative vaccine, formulated with either a squalene oil-in-water emulsion or a synthetic CpG oligodeoxynucleotide adjuvant, demonstrated efficacy in a golden Syrian Hamster challenge model. The Beta VLP vaccine induced a robust humoral immune response, with serum exhibiting neutralization not only against SARS-CoV-2 Beta but also cross-neutralizing Delta and Omicron pseudoviruses. Protective efficacy was demonstrated, evidenced by reduced viral RNA copies and mitigated weight loss and lung damage compared to controls. This compelling data instills confidence in the creation of a versatile platform for the local manufacturing of potential pan-sarbecovirus vaccines, against evolving viral threats.

1. Introduction

Both the severe acute respiratory syndrome (SARS) coronaviruses SARS-CoV and SARS-CoV-2, from the betacoronavirus genus, (subgenus sarbecovirus) have caused epidemics or pandemics in humans with profound repercussions over the last two decades [1]. The recent SARS-CoV-2 outbreak has spurred global vaccine development, pushed the frontiers of novel technologies and emphasized the need for preparedness against future zoonotic sarbecovirus pandemics. The focus also underscores the imperative to prioritize safety, efficacy, and cost-effective scalability in developing commercially viable products, especially in developing countries.

Several approaches are being used to improve the protective efficacy against a broader range of variants, including multiple antigens from

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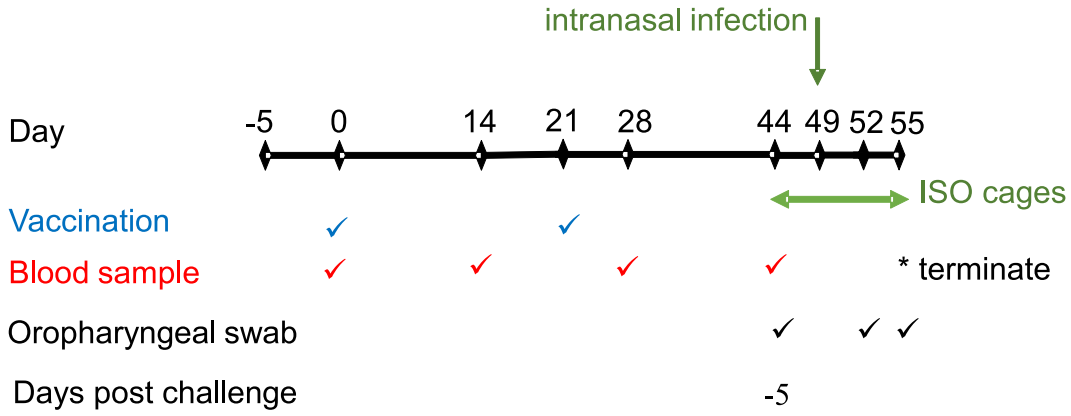
different coronavirus subgroups [2]. Our approach was to make use of virus-like particles (VLPs) as a vehicle to present the full-length spike (S) glycoprotein of SARS-CoV-2. VLPs are self-assembling bio-nanoparticles that present various epitopes on their surface, thereby mimicking the structure of native virions. These VLPs also have the advantage in vaccine development as they lack the viral genome and are therefore non-infectious [3]. Of particular interest is the plant-produced VLPs due to the low cost of manufacture compared to traditional fermenter-based alternatives, greater scalability, and intrinsic safety of production [4,5]. VLPs or proteins displayed on nanoparticles are usually more immunogenic than the unmultimerized proteins. For instance, when incorporating the capsid protein L2 of the human papillomavirus into VLPs, this led to heightened titers of cross-neutralizing antibodies compared to controls [6]. Plant-produced SARS-CoV-2 S protein based VLPs are already in clinical phase trials [7,8]. Health Canada has granted approval to Medicago and GlaxoSmithKline (GSK) for Covifenz®, a plant-derived COVID-19 VLP vaccine based on a variant of the USA Covid-19 strain. Covifenz® demonstrated good neutralizing antibodies (nAbs) with a geometric mean titre (GMT) of 172, elicited by a prime-boost vaccination approach [7]. We developed a unique prototype based on the Beta VOC and already demonstrated that these SARS-CoV-2 VLPs elicited robust nAb titres in New Zealand white rabbits, which neutralised not only Beta variant pseudoviruses but also cross-neutralised Delta (B.1.617.2) and Omicron (BA.1) pseudoviruses [9]. Here, the protective efficacy of this rSARS-CoV-2 prototype based on the Beta VOC administered with either a squalene oil-in-water based adjuvant or a CpG based adjuvant was demonstrated in a prime-boost regimen in a golden Syrian hamster challenge model.

2. Materials and methods

2.1. Ethical statement for vaccine production and laboratory animal care and use

The cloning, production and formulation of the vaccines were done in a BSL-1 laboratory under the approval of the CSIR Research Ethics Committee (Reference number 251/2018). Immunization and efficacy in Golden Syrian Hamsters under ABSL-3 conditions were approved by CSIR REC 395/2022, University of Cape Town Animal ethics committee (FHS AEC NO. 021_005), and Department of Agriculture, Land Reform

A



B

Group	Test item	Adjuvant	Dose on Day 0 and Day 21	Animal numbers of sexes	Challenge with 10^5 pfu Beta variant of SARS-CoV-2
1	SARS-CoV-2 Beta VLP	SEPIVAC SWE™	5 µg	3 male, 3 female	√
2	SARS-CoV-2 Beta VLP	DCA NADA	5 µg	3 male, 3 female	√
3	Unvaccinated/ unchallenged control	-	-	2 male, 2 female	X
4	PBS	SEPIVAC SWE™	-	2 male, 1 female	√
5	PBS	DCA NADA	-	2 male, 1 female	√

Fig. 1. (A) Schematic representation of Syrian hamster challenge study of SARS-CoV-2. (B) Vaccination and challenge schedule for the experimental and control groups of hamsters.

and Rural Development section 20 permit (12/11/1/7 (1975 RJ)).

2.2. Vaccine preparation

The plant expression vector pEAQ-HT harboring the Beta variant hCoV-19/South Africa/Tygerberg-461/2020 facilitated the transient expression and assembly of VLPs in *N. benthamiana* ΔXT/FT plant leaf tissue (Fig. S1) and characterised for vaccine formulation as previously described [9,10]. Trehalose dihydrate (Sigma-Aldrich) (15 % m/v) was added as stabiliser to partially purified VLPs prior to filter sterilisation. The VLPs were adjuvanted with either a Squalene based oil-in-water emulsion (SEPIVAC SWE™, sourced from Seppic, France), or a CpG based adjuvant (DCA NADA, a kind gift from Disease Control Africa, South Africa) in a 1:1 ratio (VLPs: adjuvant), immediately before vaccination.

2.3. Vaccination and intranasal infection in Syrian hamsters

Vaccination and challenge trials were performed on Golden Syrian hamsters aged between 6 and 9 weeks. The isolation and preparation of the Beta variant inoculum are described in the Supplementary Materials and Methods.

The vaccine challenge study was conducted as indicated in Fig. 1A and B, using 3–6 hamsters per experimental group. Hamsters in groups 1 and 2 (n = 6) were inoculated intramuscularly (i.m.) with 5 μg of Beta SARS-CoV-2 virus-like particles, formulated 1:1 in SEPIVAC SWE™, (Seppic, France) group 1, (final dose: 5 μg of VLPs in 50 μl phosphate-buffered saline (PBS) plus 50 μl SEPIVAC SWE™ emulsion) or the adjuvant DCA NADA (Disease Control Africa, South Africa) group 2, (final dose: 5 μg of VLP in 50 μl PBS plus 5 μg of DCA NADA in 50 μl) respectively, on days 0 and 21. Hamsters in group 3 (n = 4) were included as an unvaccinated and unchallenged control group. Hamsters in groups 4 and 5 (n = 3) were inoculated i.m. with SEPIVAC SWE™ adjuvant or DCA NADA adjuvant in PBS respectively, on days 0 and 21. Blood was drawn from the cranial vena cava on day 0, 14, 28 and 44.

On day 44, vaccinated animals were moved to the ABSL3 laboratory's IsoRAT900 biocontainment system, where they were intranasally infected on day 49 with 10^5 PFU of the Beta variant of SARS-CoV-2. Oropharyngeal swabs were taken on days 44 (before infection), 52 (3 days post infection), and 55 (5 days post infection). The hamsters' weights were recorded before vaccination and infection, and subsequently tracked daily after infection until the end of the experiment. The study concluded on day 55, at which point lung samples were collected in 10 % buffered formalin for histopathology testing.

2.4. Evaluation of immune responses and neutralizing antibody titres

A competitive SARS-CoV-2 ELISA containing an antigen coating of the SARS-CoV-2 Receptor Binding Domain (RBD) (Invitrogen, BMS2326) was used to measure antigen specific antibody titres of serum samples as per the manufacturer's guidelines and detailed in the Supplementary Materials and Methods.

Lentiviral pseudovirus production and neutralisation assays were performed as previously described [9] with a summary provided in the Supplementary Materials and Methods.

2.5. Determination of SARS-CoV-2 viral RNA copies

SARS-CoV-2 viral RNA copies were quantified from nasal swabs and detailed in the Supplementary Materials and Methods.

2.6. Histopathology

Lung samples were cut into sections and subjected to hematoxylin and eosin (H&E) staining for histopathological analysis and further details provided in the Supplementary Materials and Methods.

2.7. Statistical analysis

Statistical analyses were performed using GraphPad Prism version 5 software (GraphPad Software, Inc., CA, USA). Statistical significance was determined using ANOVA for multiple comparisons and the student's *t*-test was used to compare two groups. Statistical significances are indicated for values of ($p < 0.1$, 0.05, 0.001).

3. Results

3.1. Adjuvanted plant-produced SARS-CoV-2 Beta VLP vaccine induced the production of neutralizing antibodies in the golden Syrian hamster model

Serum samples collected from hamsters two weeks post-immunization were analyzed on days 14 and 44 as previously done [5], to assess SARS-CoV-2 antibody levels targeting the receptor-binding domain (RBD), (Fig. 2A, B). The adjuvanted SARS-CoV-2 Beta variant VLP groups exhibited antibody (Ab) development after the initial vaccination on day 14, with group 1 showing 50 % (male) and 53 % (female) inhibition and group 2 a 49 % (male) and 55 % (female) inhibition. Post-booster vaccination on day 44, group 1 exhibited 58 % (male) and 82 % (female) inhibition, while group 2 showed 54 % (male) and 84 % (female) inhibition. Notably, females trended higher Abs levels than males, with significant neutralization activity in female groups between days 14 and 44 ($p < 0.001$).

Assessing neutralizing assay titers (ID50) across Beta, Delta, and Omicron BA.1 (Fig. 2C, Table S1) variants from the sera revealed substantial increases after booster vaccinations in both test groups. Hamster sera for both plant-produced VLP vaccine groups (1 and 2) neutralized Beta pseudoviruses on day 14 (GMT 143 ± 117) for group 1 and (GMT = 117 ± 302) for group 2 with high variance in the titres between the individual subjects. These titres increased after the booster vaccination to GMT 583 ± 1523 for group 1 and GMT = 1231 ± 1479 for group 2. The two vaccinated groups were not significantly different from each other but differed substantially compared to their control counterparts ($p < 0.01$ and $p < 0.001$, respectively). Both the vaccinated hamster groups' antisera produced neutralization titres after the second booster, for Delta and Omicron variants as well. Notably, females in both vaccine groups exhibited higher neutralization titers than males, albeit not statistically significant (Table S1).

3.2. Adjuvanted plant-produced SARS-CoV-2 Beta VLP vaccine reduced viral RNA copies in the lungs of vaccinated hamsters

Percentage weight loss served as a disease severity indicator (Fig. 2D). Comparing the untreated control group with two vaccinated groups, group 1 exhibited significant differences on days 2 ($p < 0.05$), 3, and 5 ($p < 0.01$), indicating superior protection with the DCA NADA adjuvanted vaccine with no significant differences towards the control but with significant differences between group 1 and group 2 ($p < 0.1$, 0.05, 0.001, respectively). Both vaccine groups had values significantly different towards their respective controls, indicative of protective immunity.

At 5 days post challenge, all the animals were humanely euthanized, and oropharyngeal swabs and lung tissue samples collected to determine viral RNA copies and assess the histopathology. Multiplex qRT-PCR was done to determine the viral copies/μl collected by oropharyngeal swabs in all the test groups (Fig. 2e). A statistically significant difference between group 2 and its control, group 5 (7.8×10^4 copies/μl vs 17.3×10^4 copies/μl, $p < 0.05$) was observed on day 3 after infection but not for group 1 to its control (5.7×10^4 copies/μl vs 7.6×10^4 copies/μl), confirming the observation that group 2 had protective efficacy superior to group 1. A decrease in the viral RNA copies was however observed for both the vaccinated groups over time.

Histopathological changes consistent with acute to subacute

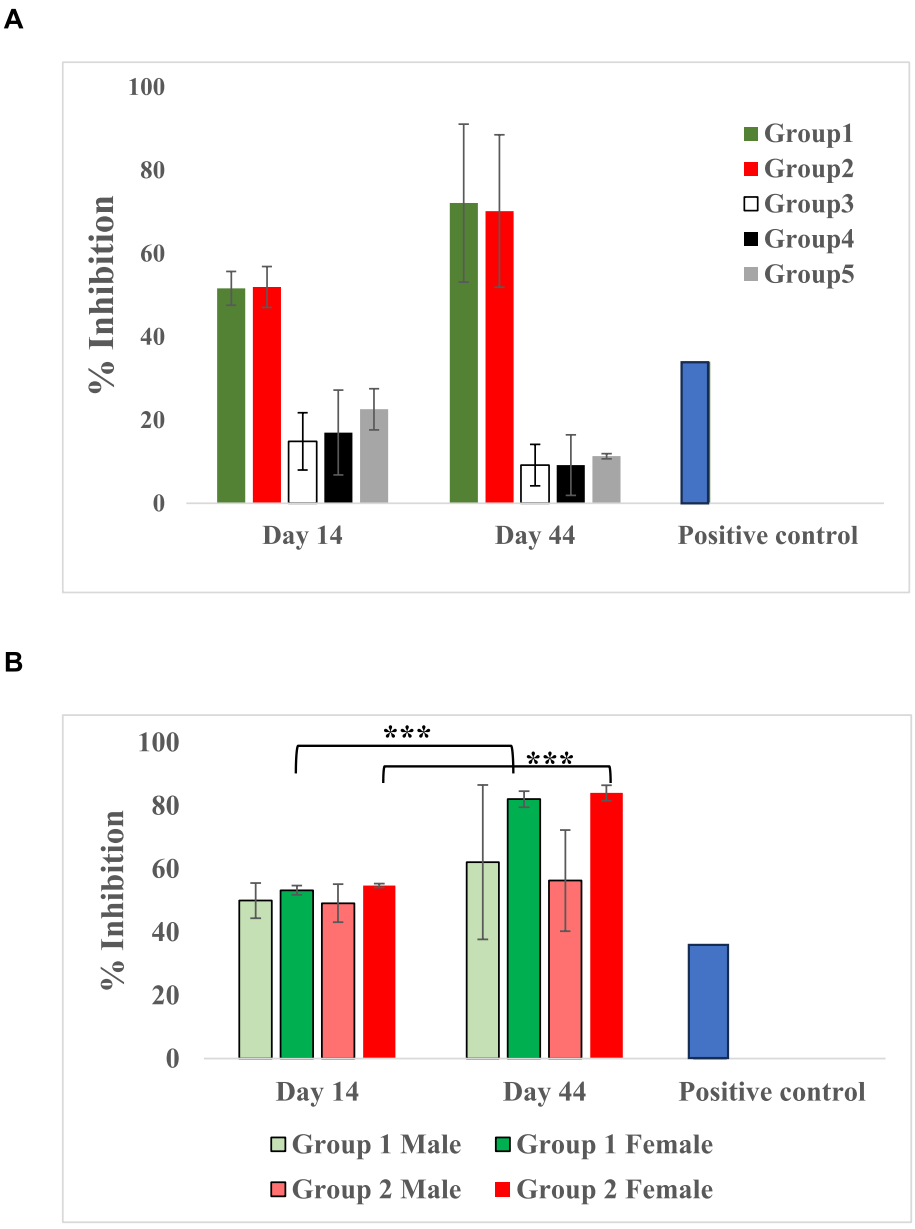


Fig. 2. Prime-boost immunization with plant-generated VLPs, with adjuvants SEPIVAC SWE™ (group 1) or DCA NADA (group 2) against SARS-CoV-2 infection. Unchallenged control n = 4 (group 3), PBS controls n = 3 (groups 4 & 5), Test groups n = 6 (groups 1–2). (**: $p < 0.01$, ***: $p < 0.001$). (A) Antibodies targeting the receptor-binding domain (RBD) were assessed within serum samples from hamsters. This evaluation was performed utilizing a commercially available competitive SARS-CoV-2 ELISA kit with a positive control provided. The recorded signals exhibited an inverse correlation with the extent of antibody inhibition and were quantified as a percentage, with a threshold of ≥30 % indicative of a positive outcome. The control groups were below the positive margin. (B) Male vs female titres for both the vaccinated groups are presented separately. (C) The neutralization titers (ID50) of serum samples. The ID50 values, presented in Log10, correspond to the dilutions at which a 50 % neutralization threshold is achieved. Two-way ANOVA, with Bonferroni post-test was used. (D) Percentage body weight change following intranasal challenge with SARS-CoV-2 Beta variant. Data were presented as mean ± SD. Day: Days post infection. (E) Following exposure to the Beta variant, qRT-PCR assessment from oropharyngeal swabs was done. Individual data points presented. One animal from group 5 on day 3 was excluded as the sample was compromised. (F) Pathological damage to the lungs in hamsters at day 49 with representative micrographs of all the test groups (scale bars 0.5 mm). (G) H&E-stained sections of lungs from virus-challenged hamsters scores based on severity, absent = 0, minimal = 1, mild = 2, moderate = 3, marked = 4 or severe = 5. Data in histogram presented as mean ± SD.

inflammation (i.e. pneumonia) were observed in several lung specimens (Fig. 2F) and scored based on levels of severity from 0 to 5 (Fig. 2G). The lung pathological damage caused by the challenge virus in the Beta VLP vaccinated groups (1 and 2) ranged from absent to marked and for the adjuvanted PBS control groups (4 and 5) the damage ranged from moderate to severe, demonstrating the protective efficacy induced by the adjuvanted plant-produced VLP vaccine. Both VLP vaccinated groups of hamsters had less pathology (lower scores) than that of the control groups (group 4 with 4.7, group 5 with 3.3) indicating that the Beta VLP vaccines provided protection against disease.

4. Discussion

The continuous emergence of new SARS-CoV-2 variants worldwide has spurred innovation in vaccine development. Safety, efficacy, and cost-effective scalability is especially crucial for low-to-middle income

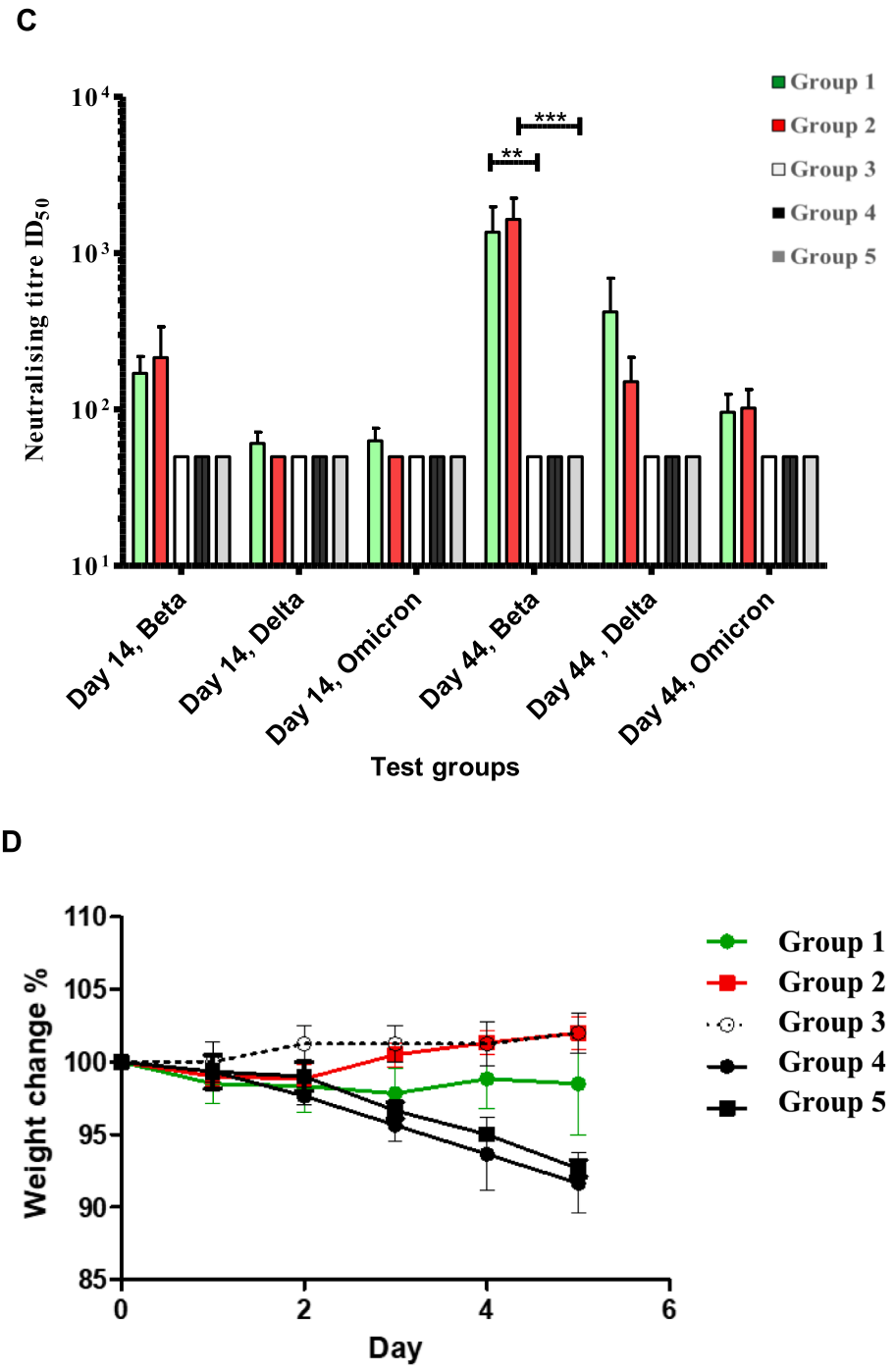


Fig. 2. (*continued*).

countries (LMICs) with cold chain logistics challenges, and therefore key considerations. Plant-derived vaccines, exemplified by clinical trials on a recombinant quadrivalent VLPs (QVLP) influenza vaccine [11], rotavirus VLPs [12], and COVID-19 VLPs [7], have proven safe and immunogenic for human use. Notably, Kentucky Bioprocessing and Baiya Phytopharm Co., Ltd actively contribute to ongoing plant-derived COVID-19 vaccine development [13].

This study, employing a hamster model for its lung-related clinical features akin to humans post-SARS-CoV-2 exposure, demonstrated the vaccine's ability to elicit a robust immune response with an adjuvanted 5 μg VLP dosage. Hamsters developed antibodies to the RBD post-immunization, significantly boosted upon the second dose, resulting in high titers of neutralizing antibodies, indicative of robust immune protection against symptomatic SARS-CoV-2 infection, aligning with previous research of Khoury et al. (2021) and Dimeglio et al. (2022), these elevated levels of neutralizing antibodies serve as robust predictors of immune safeguarding against symptomatic SARS-CoV-2 infection [14,15].

In this study, Beta VLPs adjuvanted with SEPIVAC SWE™ and DCA NADA both resulted in protective immunity, with the CpG-based adjuvant proving superior. CpGs are ligands of Toll-like receptor 9 (TLR9), boost host CD4+ T cell and humoral immune responses, conferring protection against virus challenges [16]. Similarly, Wang et al. (2022), demonstrated that a CpG/alum adjuvant enhanced the immunogenicity of a SARS-CoV-2 antigen vaccine [17].

Gender comparison within test groups revealed significantly higher

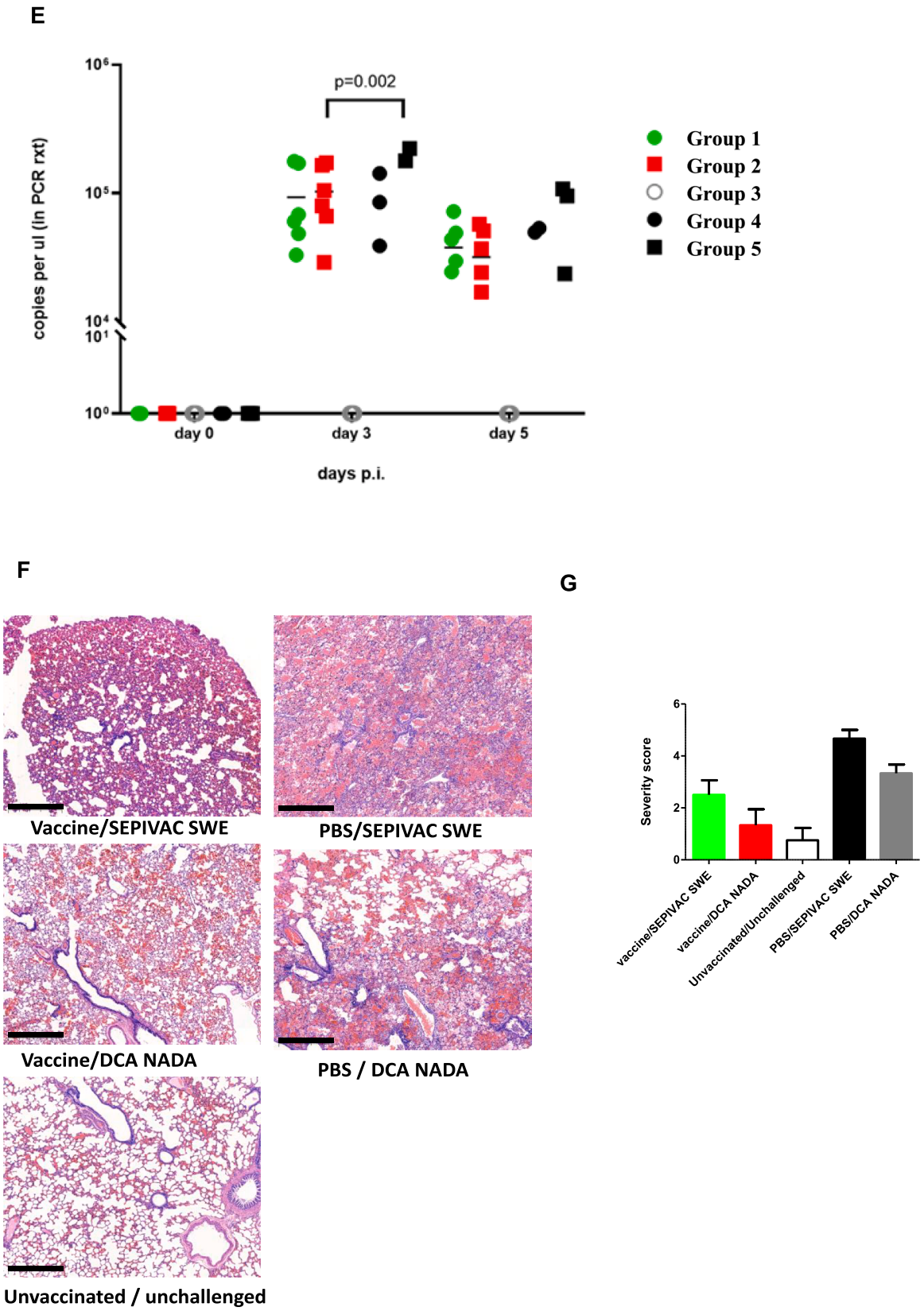


Fig. 2. (*continued*).

neutralizing and anti-RBD antibodies in females, particularly post-booster dose. The mechanism behind this observed biological sex difference requires further investigation, encompassing genetics, microbiome composition, metabolic dynamics, and the aging process [18–20].

In conclusion, the study demonstrated that a prime-boost regimen with a plant-derived Beta VLP vaccine adjuvanted with SEPIVAC SWE™ or DCA NADA was well-tolerated, highly immunogenic, and cross-reactive to Delta and Omicron pseudovirus variants. The plant-based

platform presents a promising avenue for low-cost, scalable COVID-19 and potential pan-sarbecovirus vaccines production, addressing the evolving challenges of the ongoing pandemic.

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CRediT authorship contribution statement

Yolandy Lemmer: Conceptualization, Data curation, Formal analysis, Methodology, Validation, Writing – original draft, Writing – review & editing. **Ros Chapman:** Data curation, Formal analysis, Methodology, Resources, Validation, Writing – review & editing. **Celia Abolnik:** Conceptualization, Methodology. **Tanja Smith:** Formal analysis, Methodology, Writing – review & editing. **Georgia Schäfer:** Formal analysis, Methodology, Writing – review & editing. **Tandile Hermanus:** Methodology. **Ilse du Preez:** Methodology. **Kruger Goosen:** Resources. **Kamogelo M. Sepotokele:** Methodology. **Sophette Gers:** Data curation, Formal analysis, Methodology. **Tasnim Suliman:** Methodology. **Wolfgang Preiser:** Resources, Writing – review & editing. **Megan L. Shaw:** Resources, Writing – review & editing. **Robyn Roth:** Methodology. **Alma Truyts:** Methodology. **John Chipangura:** Resources, Methodology. **Martin Magwaza:** Conceptualization, Funding acquisition, Resources, Writing – review & editing. **Osborn Mahanjana:** Conceptualization, Funding acquisition, Resources, Writing – review & editing. **Penny L. Moore:** Validation, Writing – review & editing. **Martha M. O'Kennedy:** Conceptualization, Data curation, Formal analysis, Methodology, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Additional data other than what is presented in the manuscript can be requested from the corresponding author.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2024.01.036.

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