THE LANCET
Infectious Diseases

Supplementary appendix

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Supplement to: Vacharathit V, Aiewsakun P, Manopwisedjaroen S, et al. CoronaVac induces lower neutralising activity against variants of concern than natural infection. *Lancet Infect Dis* 2021; published online August 26. https://doi.org/10.1016/S1473-3099(21)00568-5.

Supplementary Appendix

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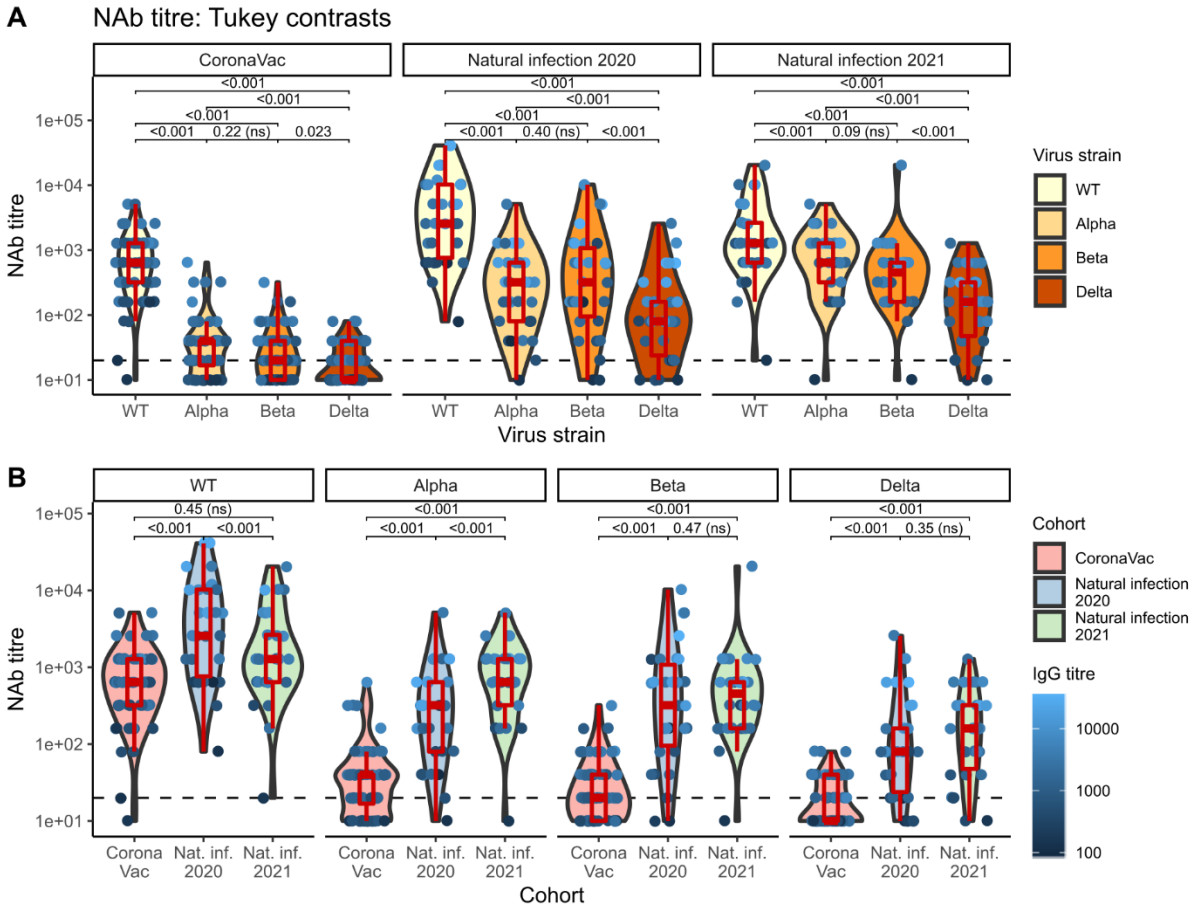
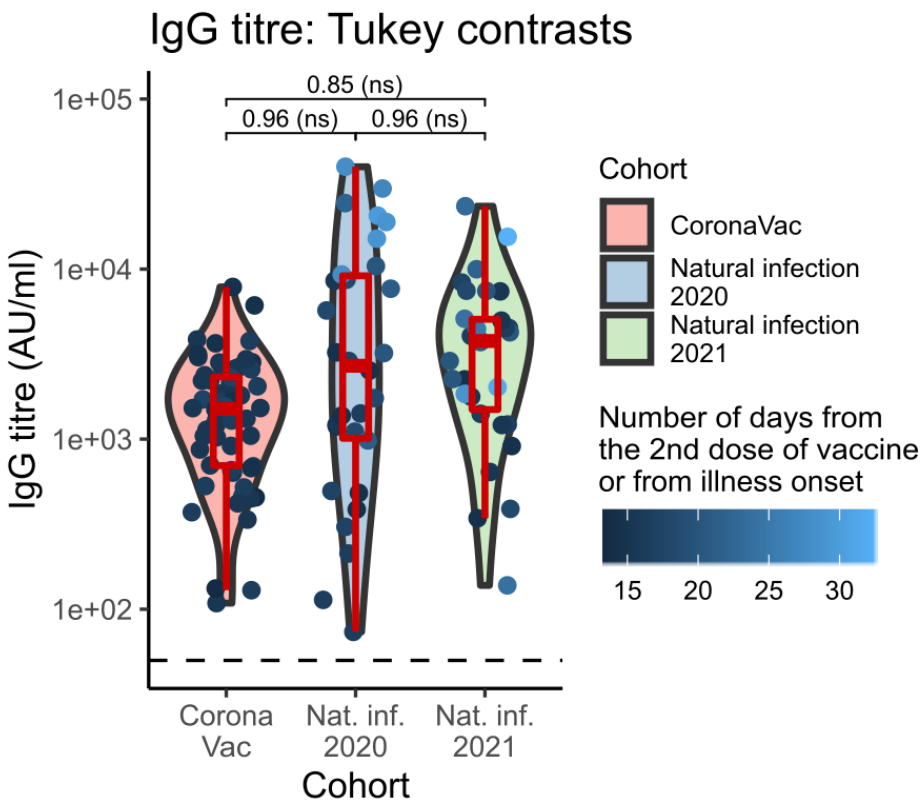


Supplementary Figure 1. Violin plots of NAb titres grouped by virus strain nested within cohort groups (top), and by cohort group nested within virus strain (bottom). Boxplots indicate medians and IQRs. The horizontal dotted line indicates the threshold for positive detection (20 units). The data points are coloured according to the IgG titre. Numbers above the violin plots are Tukey-adjusted p-values; 'ns' = non-significant. IgG titre-, sex-, and age-adjusted geometric mean NAb titres can be found in **Supplemental Table 3**.



Supplementary Figure 2. Violin plots of IgG titre by cohort group. Boxplots indicate medians and IQRs. The horizontal dotted line indicates the threshold for positive detection (50 AU/ml). There was no significant difference in SARS-CoV-2 S1-RBD-binding IgG levels between groups adjusting for patients' sex, age, and serum collection date. Examination of the model revealed that age and sex did not significantly affect IgG titre, whereas serum collection date did. Numbers above the violin plots are Tukey-adjusted p-values; 'ns' = non-significant. Geometric mean IgG titre (95% CI) computed at mean serum collection date (ie, mean number of days since last vaccine dose or illness onset; 17.16 days, standard deviation = 4.26 days), age (42.25 yr, standard deviation = 13.7 yr) averaged cross sexes: CoronaVac = 1741.28 (1240.48-2444.25) AU/ml; Natural infection 2020 = 1875.07 (1236.29-2843.89) AU/ml; Natural infection 2020 = 2034.54 (1340.47-3087.99) AU/ml. The data points are colored according to the serum collection date.

Supplementary Tables

Characteristic		CoronaVac	Natural infection 2020	Natural infection 2021	P-value ‡	
					Log_{10}(IgG titre) covariate	**Log_{10}(NAb titre) covariate**
Sample – no.		60	30	30		<0.001
Age – median (IQR*)		35 (29.75–44.50)	44.80 (36.80–55.85)	46.45 (34.45–53.00)	0.41	0. 68
Female – no. (%)		51 (85%)	11 (37%)	11 (37%)	0.68	0.013
Days from latest vaccine dose or from illness onset – median (IQR*)		15 (15–15.25)	17.50 (15.25–21)	17.50 (15–21.75)	<0.001	
IgG titre – median (IQR*)		1508.4 (698.8–2320.1)	2706.9 (1013.7–9139.5)	3777.2 (1500.8–5068.8)		<0.001
Neutralizing antibody titre† – median (IQR*)	**WT**	640 (320–1280)	2560 (800–10240)	1280 (640–2635)		
	Alpha	40 (17.5–40)	320 (80–640)	640 (320–1280)		
	Beta	20 (10–40)	320 (100–1120)	480 (160–640)		
	Delta	10 (10–40)	80 (25–160)	160 (50–320)		

Supplementary Table 1. Cohort demographics of CoronaVac vaccinees as well as naturally infected patients hospitalized in 2020 and 2021 who were included in the study.

*IQR denotes inter-quartile range

†Neutralizing antibody titres below the limit of detection (<10) were assigned a value of 10

‡See the models in the **Supplementary methods**

NAb Titre ≥ 20	WT	Alpha	Beta	Delta
CoronaVac	59/60 (98.33%)	45/60 (75.00%)	42/60 (70.00%)	29/60 (48.33%)
Natural infection 2020	30/30 (100.00%)	29/30 (96.67%)	28/30 (93.33%)	26/30 (86.67%)
Natural infection 2021	30/30 (100.00%)	29/30 (96.67%)	29/30 (96.67%)	28/30 (93.33%)

Supplementary Table 2. Percentages of participants with NAb titres ≥20 (NAb positivity cut-off) against WT, Alpha, Beta and Delta strains within each cohort.

Cohort	Virus strain	NAb titre		
		Geometric mean	Lower 95% CI	Upper 95% CI
CoronaVac	WT	774.48	607.22	987.82
	Alpha	44.64	35	56.94
	Beta	35.03	27.46	44.68
	Delta	24.48	19.2	31.23
Natural Infection 2020	WT	2329.83	1696.56	3199.49
	Alpha	178.31	129.84	244.86
	Beta	235.03	171.15	322.76
	Delta	69.15	50.35	94.96
Natural Infection 2021	WT	997.2	725.47	1370.72
	Alpha	464.62	338.01	638.65
	Beta	306.53	223	421.35
	Delta	94.35	68.64	129.69

Supplementary Table 3. Geometric mean NAb titre values, computed at mean age (42.25 yr, standard deviation = 13.7 yr), geometric mean values of IgG titre (1824.71 AU/ml, geometric standard deviation = 3.46 AU/ml), and averaged across the sexes. The computation assumed a linear mixed model, in which the effects of participants' gender, age, (log-10 transformed) IgG titre, virus strain, and immunogenic elicitor on the observed values of (log-10 transformed) NAb titre were treated as fixed effects, and the effect of subject sampling was treated as a random effect. The model allowed the effects of virus strain and immunogenic elicitor to vary independently among each combination. See model specification in Supplementary Methods - Data and statistical analyses

Supplementary methods

Ethics Statement

All methods were performed following standard protocols approved by the institutional review committee. Written informed consent was obtained and the study protocol and human ethics were approved by the Human Research Ethics Committee, Faculty of Medicine Ramathibodi Hospital (COA. MURA2021/264) and the Ethics Committee of National Cancer Institute, Thailand (EC COA 019/2021).

Virus variants and culture

The wildtype SARS-CoV-2 virus (SARS-CoV-2/human/THA/LJ07_P3/2020), was isolated from a nasopharyngeal swab sample from an RT-PCR confirmed COVID-19 patient provided by Bamrasnaradura Infectious Diseases Institute, Nonthaburi, Thailand. African green monkey (Cercopithecus aethiops) kidney epithelial cells (Vero cells) (ATCC CCL-81) were used for virus isolation and a Vero cell derivative (Vero E6 cells) (ATCC CRL-1586) was used for virus propagation.

The B.1.1.7 (SARS-CoV-2/human/THA/NH657_P3/2021) and B.1.617.2 (SARS-CoV2/human/THA/OTV007_P3/2021) variants were isolated from nasopharyngeal swab samples from RT-PCR confirmed COVID-19 patients provided by Ramathibodi Chakri Naruebodindra Hospital (Chakri Naruebodindra Medical Institute), Samut Prakan, Thailand. The B.1.351 variant (SARS-CoV-2/human/THA/NH088_P3/2021) was isolated from a nasopharyngeal swab sample from an RT-PCR confirmed COVID-19 patient provided by the Division of Genomic Medicine and Innovation Support, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand. For B.1.1.7, B.1.617.2 and B.1.351, Vero E6 cells were used for both virus isolation and propagation. Vero cells were cultured in minimum essential medium (MEM) (Gibco, Detroit, MI, USA) and Vero E6 were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco). Virus isolation and propagation were conducted

in a certified BSL3 facility at the Microbiology Department, Faculty of Science, Mahidol University, Bangkok, Thailand.

Sequencing

Nucleic acid was extracted from 200 µl culture supernatant using the GenTiTM32 Automatic Extraction System (Advanced Viral DNA/RNA Extraction Kit) according to the manufacturer's instructions, followed by library preparation using the ARTIC SARS-CoV-2 sequencing protocol (https://dx.doi.org/10.17504/protocols.io.bgxjjxkn). Prepared libraries were sequenced (paired-end) with single indexing on a MiSeq (Illumina) sequencer at the Center of Medical Genomics, Ramathibodi Hospital, Thailand. The sequence of a reference SARS-CoV-2 (NCBI Reference Sequence: NC_045512.2) was used as a query sequence to pull SARS-CoV-2 reads from the Next Generation Sequencing read datasets by BLASTn. The BLASTn results were then used to construct alignments of the reads. After manual curation, the consensus sequences were determined according to the top 80% most frequent nucleotide residues at each position.

The consensus sequences of the spike protein of the wildtype, B.1.1.7, B.1.617.2 and B.1.351 have been deposited in GenBank and can be accessed under the accession numbers: SARS-CoV-2/human/THA/LJ07_P3/2020 MZ815437, SARS-CoV-2/human/THA/NH657_P3/2021 MZ815438, SARS-CoV-2/human/THA/OTV007_P3/2021 MZ815439, and SARS-CoV-2/human/THA/NH088_P3/2021 MZ815440, respectively.

Cohort

Patients were confirmed to be infected by RT-PCR on nasopharyngeal and throat swab specimens through amplification of SARS-CoV-2 ORF1AB and N target gene fragments (Sansure Biotech Inc, Changsha, PR China).

Live-virus microneutralization

Sera were heat inactivated at 56ºC for 30 min then two-fold serially diluted starting from 1:10. Equal volumes of SARS-CoV-2 were spiked into the serial dilutions at an infectious dose of 100 $TCID_{50}$ (50% tissue culture infectious dose) and incubated for 1h at 37ºC. Vero E6 cells (ATCC USA) were pre-seeded in Dulbecco's modified Eagle medium (DMEM) supplemented with 2% fetal bovine serum (FBS), 100 U/mL penicillin, and 0.1 mg/mL of streptomycin. 100 µL of the virus-serum mixtures at different dilutions were added to 1×10^4 pre-seeded Vero E6 cell monolayers in duplicate on a 96-well microtiter plate, then incubated for 2 days at 37ºC and 5% CO_2. The last two columns contained the virus control, cell control, and virus back-titration. Medium was discarded and cells were fixed and permeabilized with ice-cold 1:1 methanol/acetone fixative for 20 min at 4ºC. Cells were washed thrice with 1xPBS containing 0.05% Tween 20 then blocked with a blocking buffer consisting of 2% bovine serum albumin (BSA) and 0.1% Tween 20 in 1×PBS for 1h. After washing 3 more times with wash buffer, SARS-CoV/SARS-CoV-2 nucleocapsid mAb (Sino Biological, Cat#40143-R001) diluted 1:5000 in 1×PBS containing 0.5% BSA and 0.1% Tween 20 was added to each well and incubated for 2h at 37ºC. Detection antibody was removed by washing the plate 3 more times, then 1:2000 HRP-conjugated goat anti-rabbit polyclonal antibody (Dako, Denmark A/S, Cat#P0448) was added and the plate incubated at 37ºC for 1h. Plates were washed thrice more, then TMB substrate was added (KPL, Cat#5120-0075) for 10 min. The reaction was stopped with 1N HCl. Absorbance was measured at 450 and 620 nm (reference wavelength) with an ELISA plate reader (Tecan Sunrise).

The average optical density (O.D.) at 450 and 620 nm were determined for virus control and cell control wells, and the neutralizing endpoint was determined by 50% specific signal calculation. The virus neutralizing endpoint titre of each serum was expressed as the reciprocal of the highest serum dilution with an OD value less than X which was calculated as follows:

X = [(average A450−A620 of 100×$TCID_{50}$ virus control wells)−average A450−A620 of cell control wells)]/2+average A450−A620 of cell control wells).

Sera which tested negative at 1:10 dilution were assigned a titre of <10. Sera were considered positive if neutralization titre was ≥20. Live SARS-CoV-2 viruses at passage 3 or 4 and Vero E6 cells at 20 maximum passages were employed. Activities with live viruses were carried out in a certified BSL-3 facility.

Data and statistical analyses

To test if there were significant differences among the IgG titres induced by various immunogenic elicitors (CoronaVac and natural infections) while adjusting for potential effects of participant age, sex, and serum collection date (**Supplementary Figure 2**), we first fitted a linear model to the data as shown:

$$y_{IgG[i]} = \beta_{elicitor[i]} + \beta_{date}x_{date} + \beta_{sex}x_{sex} + \beta_{age}x_{age} + e_{[i]};$$

for immunogenic elicitor i, $y_{IgG[i]}$ is (log-10 transformed) IgG titre; x_{date} is the serum collection date in days (from latest vaccine dose or from illness onset); x_{sex} indicates if the participant was a male; x_{age} is the age of the participant in years; $\beta_{elicitor[i]}$ is the effect of the elicitor, while β_{date}, β_{sex}, and β_{age} are the effects of the serum collection date, gender, and age, on the level of IgG titre respectively, and $e_{[i]}$ is the residual error term. $e_{[i]}$ are assumed to be independently and identically distributed (i.i.d.) $N(0, \sigma^2)$. The *lm* function as implemented in the R library *stats* (v 4.0.4) was used to fit the model to the data. Conditioning on the estimated linear model, the level of IgG titre among the three cohort groups were then compared using the Tukey method, adjusting for serum collection date, age, and sex, using the *emmeans* function as implemented in the R library *emmeans* (v 1.6.1).

Similarly, to examine if NAb titres were significantly different among various groups of immunogenic elicitors and virus strains tested, adjusting for participant age, sex, IgG titre level and the random effects from using shared sera to measure NAb titres (**Supplementary Figure 1**), we fitted the following linear mixed model to the data by using the *lmer* function implemented in the R library *lme4* :

$$y_{NAb[i][j][k]} = \gamma_{elicitor[i]} + \gamma_{virus[j]} + \gamma_{elicitor[i]virus[j]} + \gamma_{sex}x_{sex[k]} + \gamma_{age}x_{age[k]} + \gamma_{IgG}x_{IgG[k]} + \delta_{[k]} + e_{[i][j][k]};$$

for immunogenic elicitor i virus j and subject k, $y_{NAb[i][j][k]}$ is (log-10 transformed) NAb titre; $\gamma_{elicitor[i]}$ is the effect of elicitor, $\gamma_{virus[j]}$ is the effect of virus; $\gamma_{elicitor[i]virus[j]}$ is the effect of the interaction between the elicitor and the virus; $x_{sex[k]}$ indicates if the participant k was a male; $x_{age[k]}$ is the age of the participant k in years; $x_{IgG[k]}$ is (log-10 transformed) IgG titre of participant k; γ_{sex}, γ_{age}, and γ_{IgG} are the fixed effects of the gender, age, and IgG titre on the level of NAb titre, respectively; the varying coefficient $\delta_{[k]}$ accounts for the random effect raising form the fact that serum form a single participant k was used to measure NAb titres against the four virus strains under the study. $\delta_{[k]}$ is assumed to be normally distributed with mean zero and variant σ^2_{sample}, where σ^2_{sample} indicates the overall variation of the model intercepts among the participants, and similarly $e_{[i][j][k]} \sim i.i.d.N(0, \sigma^2)$. The interaction term between the elicitor and the virus, $\gamma_{elicitor[i]virus[j]}$, was included to allow the effects of virus strain and immunogenic elicitor on the NAb titre to vary independently among each combination. Contrasting with the first model, this model did not include serum collection date x_{date} as a covariate of NAb titre. This was because that serum collection date strongly impacted IgG titre and, based on the nature of the experiment, it was actually IgG titre that directly determined NAb titre. Therefore, serum collection date was not included in the model as it would be redundant to the inclusion of IgG titre (i.e. containing strongly correlating / the same kind of information). Based on the estimated model, NAb titre values were compared between groups using the Tukey method, similar to the IgG titre comparisons (see above).

Conflicts of interest

All authors declare no conflict of interest.

Funding

This work was supported by the National Research Council of Thailand (วช.อว.(อ)(กบท2)/332/2564 and วช.อว.(อ)(กบท2)/256/2564), the Mahidol University for Integrated and Multidisciplinary Research Cluster grant (MRC-IM 02/2564), the Program Management Unit C, and the Ramathibodi Foundation. Funding sources had no involvement in study design, data collection, analysis or interpretation, nor in the writing of the manuscript and in the decision to submit the manuscript for publication.

Acknowledgments

We would like to thank all the professionals in our COVID-19 research team and all the study participants. We also thank Dr. Man Van Minh Nguyen for helpful discussion regarding statistical analyses and Dr. Waradon Sungnak for helpful input.

Author contributions

VV drafted the manuscript and interpreted the data, PA supervised, analyzed, interpreted the data and edited the manuscript, SM, TL, SE, NL, C Setthaudom, and WS performed experiments and collected data, C Srisaowakarn supervised, performed experiments and collected data, AP was project administrator and edited the manuscript, S Srichatrapimuk, SK and PW provided clinical samples, S Sangrajrang, JR, S Sungkanuparph supervised and provided clinical samples, TI and WC acquired funding, supervised and provided clinical samples, SN was project administrator and provided clinical samples, IS and NW supervised, MK supervised, SH was project administrator and edited the manuscript, AT conceptualized the idea, was project administrator, supervised, and edited the manuscript.