Supplementary file

Article Title: Humoral Immunogenicity and Efficacy of a Single Dose of ChAdOx1 MERS Vaccine Candidate in Dromedary Camels

Authors: Naif Khalaf. Alharbi[1,2*], Ibrahim Qasim[3], Abdulrahman Almasoud[1], Haya A. Aljami[1], Mohamed W. Alenazi[1], Ali Alhafufi[3], Omar S. Aldibasi[4], Anwar M. Hashem[5,6], Samy Kasem[3,7], Raed Albrahim[3], Musaad Aldubaib[8], Ali Almansour[3], Nigel J. Temperton[9], Alexandra Kupke[10, 12], Stephan Becker[10,12], Ali Abu-obaidah[3], Ali Alkarar[3], In-Kyu Yoon[11], Esam Azhar[13,14], Teresa Lambe[15], Faisal Bayoumi[3], Ali Aldowerij[3], Osman H. Ibrahim[3], Sarah C. Gilbert[15], Hanan H. Balkhy[1,2,16]

Affiliations:

[1]Department of Infectious Disease Research, King Abdullah International Medical Research Center, Riyadh, Saudi Arabia

[2]King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia

[3]Ministry of Environment, Water and Agriculture (MEWA), Riyadh, Saudi Arabia

[4]Department of Bioinformatics and Biostatistics, King Abdullah International Medical Research Center, Riyadh, Saudi Arabia

[5]Department of Medical Microbiology and Parasitology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

[6]Vaccines and Immunotherapy Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

[7]Department of Virology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, El Geish Street, 33516, Egypt

[8]College of Agriculture and Veterinary Medicine, Qassim University, Qassim, Saudi Arabia

[9]Viral Pseudotype Unit, Medway School of Pharmacy, University of Kent, Chatham, Kent, ME4 4TB, United Kingdom

[10]Institute of Virology, Philipps University of Marburg, Marburg, Germany

[11]International Vaccine Institute, Seoul, South Korea

[12]German Center for Infection Research, TTU Emerging Infections, Germany

[13]Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

[14]Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

[15]The Jenner Institute, University of Oxford, Oxford OX3 7DQ, UK

[16]Department of Infection Prevention and Control, Ministry of National Guard - Health Affairs, Riyadh, Saudi Arabia

*To whom correspondence should be addressed: harbina2@nga.med.sa

I. Supplementary Figures

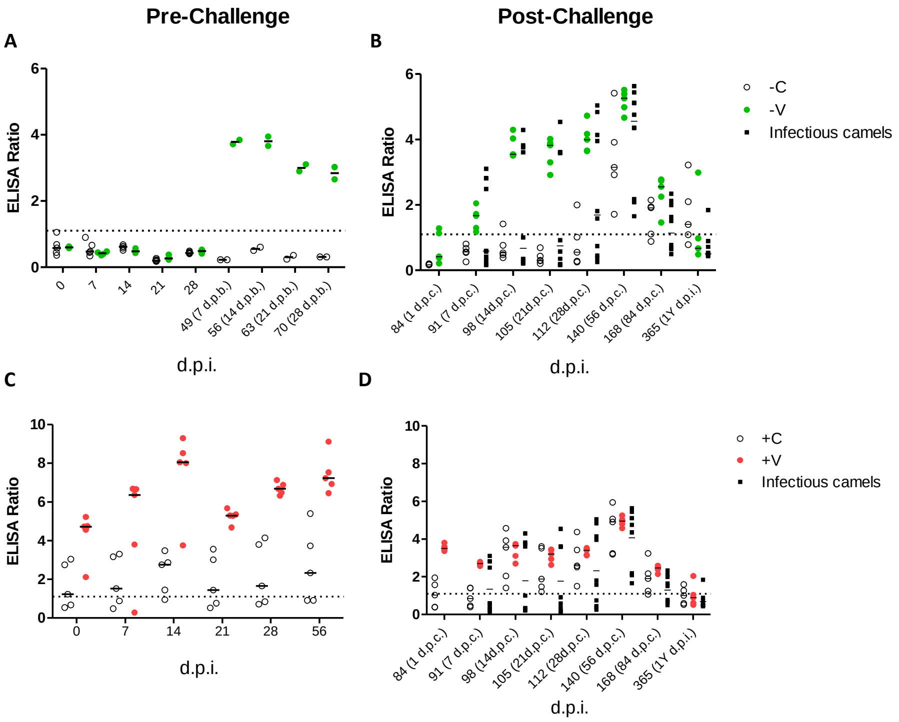


Figure S1: Antibody immune responses in dromedaries vaccinated with ChAdOx1 MERS assessed by Euroimmun ELISA kits.

Seronegative calves were either immunised with control injections (PBS or ChAdOx1 eGFP), group -C (open circles), or with ChAdOx1 MERS, group -V (green closed circles). Seropositive camels were either immunised with control injections (PBS or ChAdOx1 eGFP), group +C (open square), or with ChAdOx1 MERS, group +V (red closed square). Serum samples from different days post immunisation (d.p.i.) were evaluated in Euroimmun ELISA kits. Infectious camels used as a natural challenge models were also evaluated (black square). Data are plotted before the challenge (**A and C)** and after the challenge **(B and D)** with timepoints shown as d.p.i., days post boost (d.p.b.) and days post challenge (d.p.c.). Y: year; +: seropositive; -: seronegative; C: control group; V: vaccinated group.

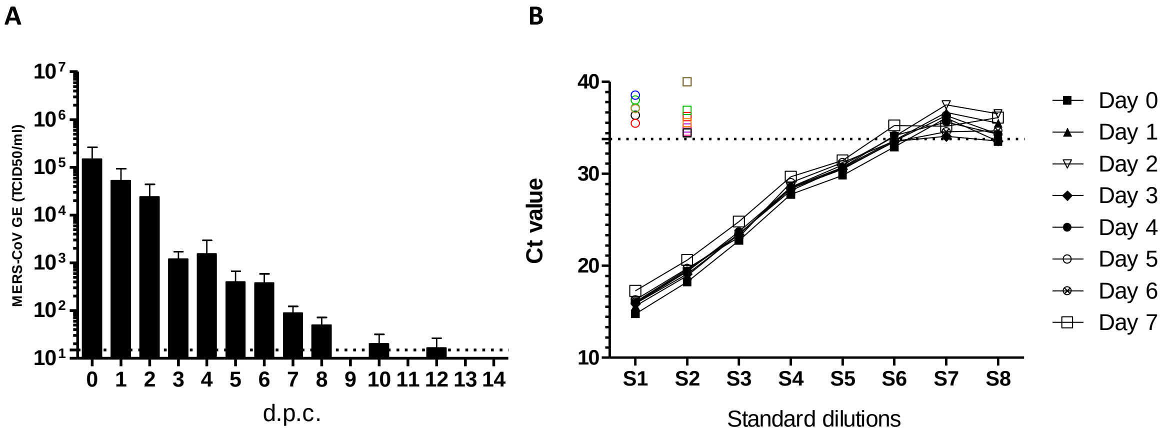


Figure S2: Genome equivalent (TCID$_{50}$/ml) titres in the MERS-CoV infectious camels.

A: Virus titres in MERS-CoV infectious camels used as a natural infection model were evaluated for 14 days post challenge (d.p.c.) using standard curve based RT-qPCR assay to report MERS-CoV GE (TCID$_{50}$/ml). Dotted line represents the detection limit of the assay, which is determined in graph **B**. **B:** Viral RNA from a sample with a known TCID$_{50}$/ml titre was utilised to set up the standard curve in RT-qPCR to evaluate the MERS-CoV GE (TCID$_{50}$/ml) titres. The detection limit of the assay was considered as the value of the last dilution before the plateau curve. Coloured circle and square symbols indicate values of negative samples from healthy camel and human, respectively.

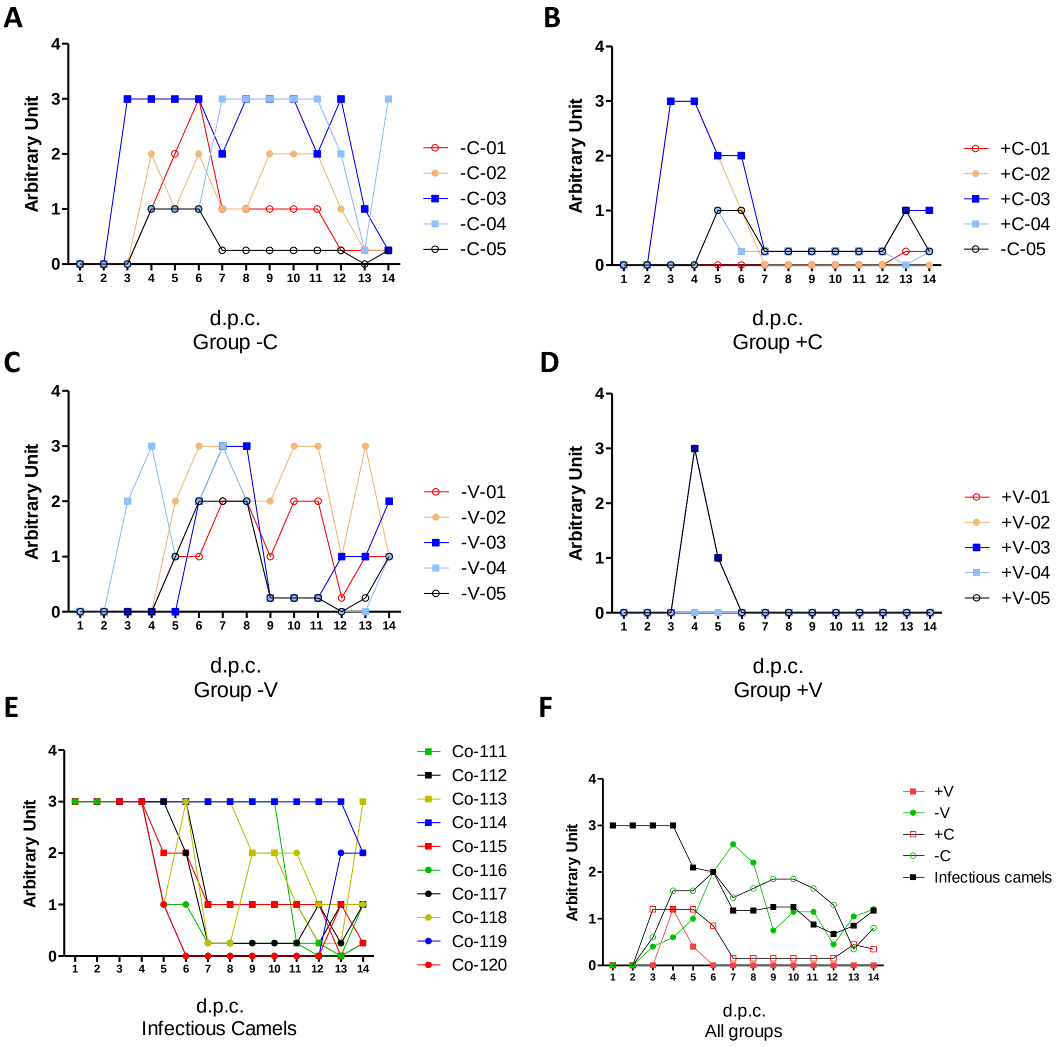
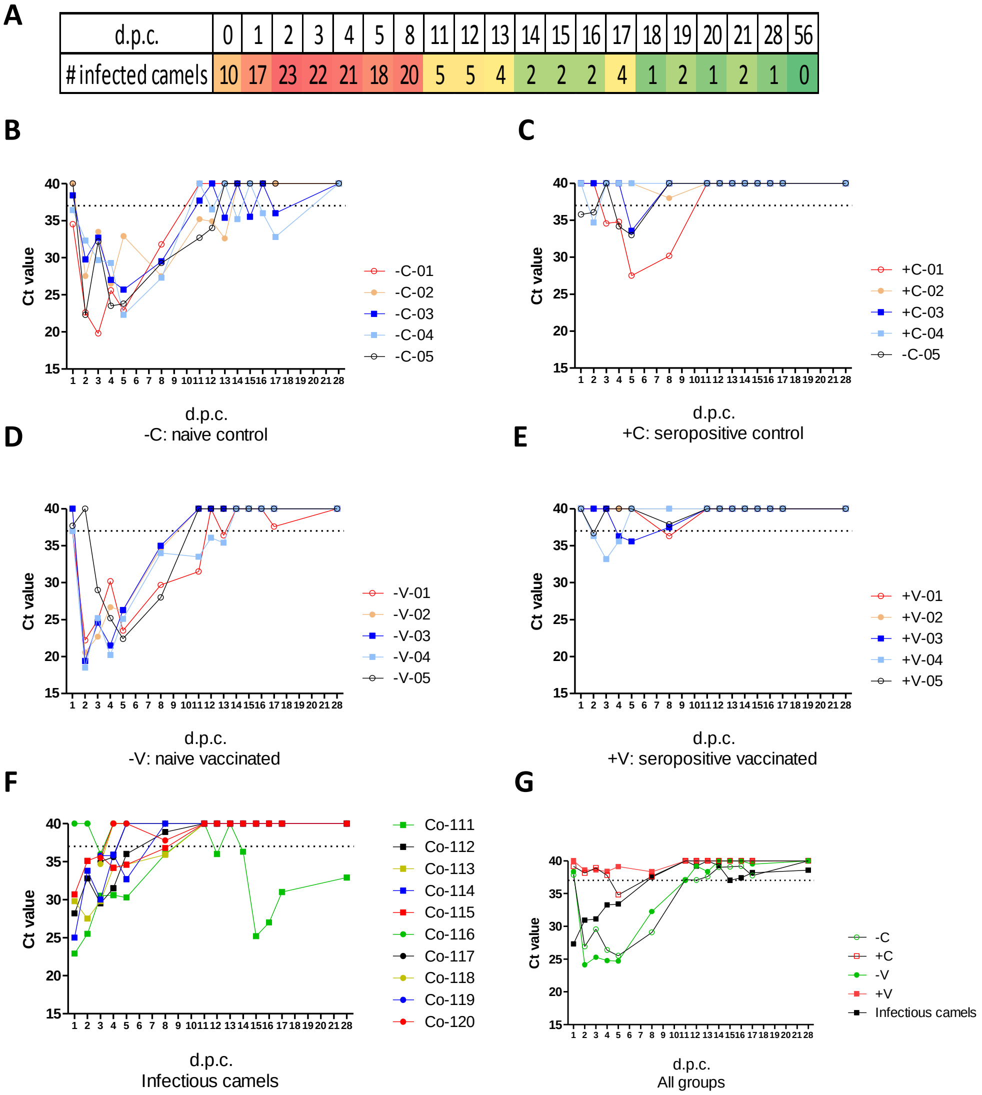


Figure S3: Severity and abundance of nasal discharge post natural infection challenge.

A-D: Scores of nasal discharge are reported for the twenty camels in all experimental groups of -C, -V, +C, and +V for 14 d.p.c. **E:** scores for the ten naturally infectious camels (used as a challenge model). **F:** average score per group. The scores were recorded as arbitrary units of 0 (normal), 0.25 (recovered), 1 (mild), 2 (moderate), or 3 (severe) depending on the nasal discharge abundance. Statistical analysis on areas under the curve of these scores is presented in Figure 3. +: seropositive; -: seronegative; C: control group; V: vaccinated group; Co-: co-housed camel.



A

d.p.c.	0	1	2	3	4	5	8	11	12	13	14	15	16	17	18	19	20	21	28	56
# infected camels	10	17	23	22	21	18	20	5	5	4	2	2	2	4	1	2	1	2	1	0

Figure S4: Efficacy of ChAdOx1 MERS in dromedaries based on RT-qPCR assay.

A: Camels in all groups (total n=20) were co-housed with naturally infectious camels (n=10), and the number of infected camels increased at different days post challenge (d.p.c.) as shown in the heatmap table. Nasal swabs were assessed for the presence of MERS-CoV UpE gene by RT-qPCR for 21 d.p.c. and for 28 d.p.c. The Ct values are shown for individual camels in each group (Groups: -C **(B)**, +C **(C)**, -V **(D)**, or +V **(E)**), the infectious camels **(F),** and as mean Ct values for each group **(G)**. +: seropositive; -: seronegative (naive); C: control group; V: vaccinated group.

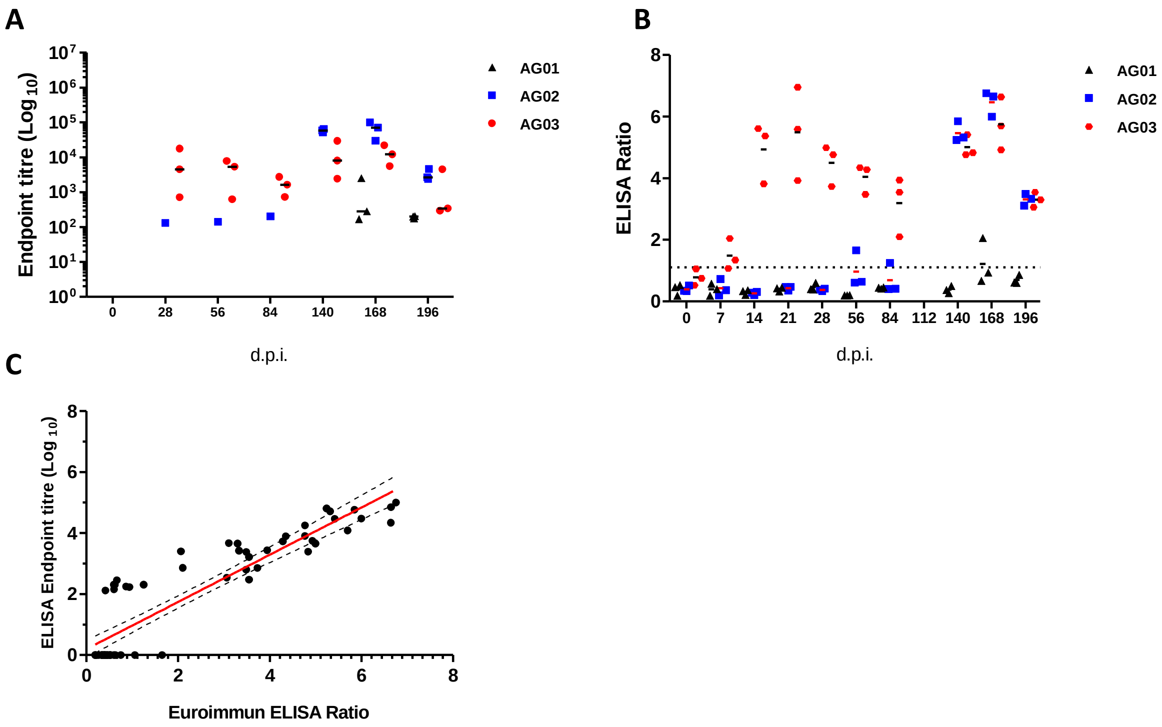
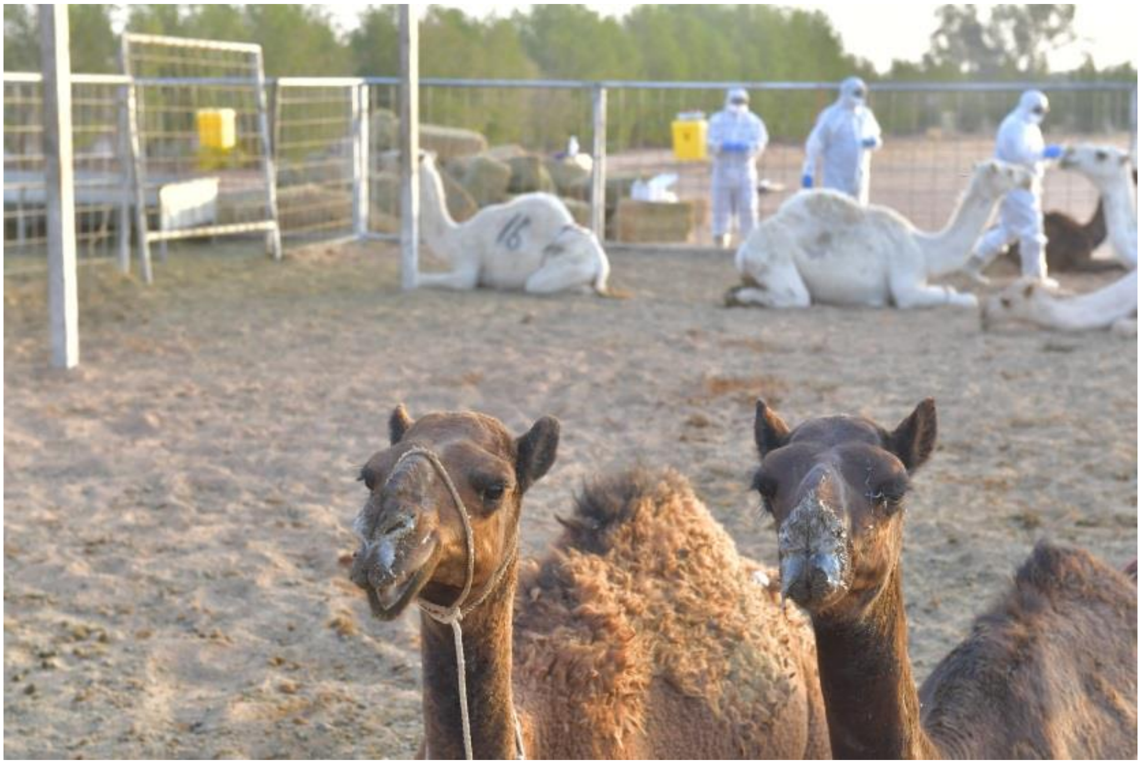


Figure S5: Antibody immune responses in different age of dromedary calves vaccinated with ChAdOx1 MERS assessed by Euroimmun ELISA kits.

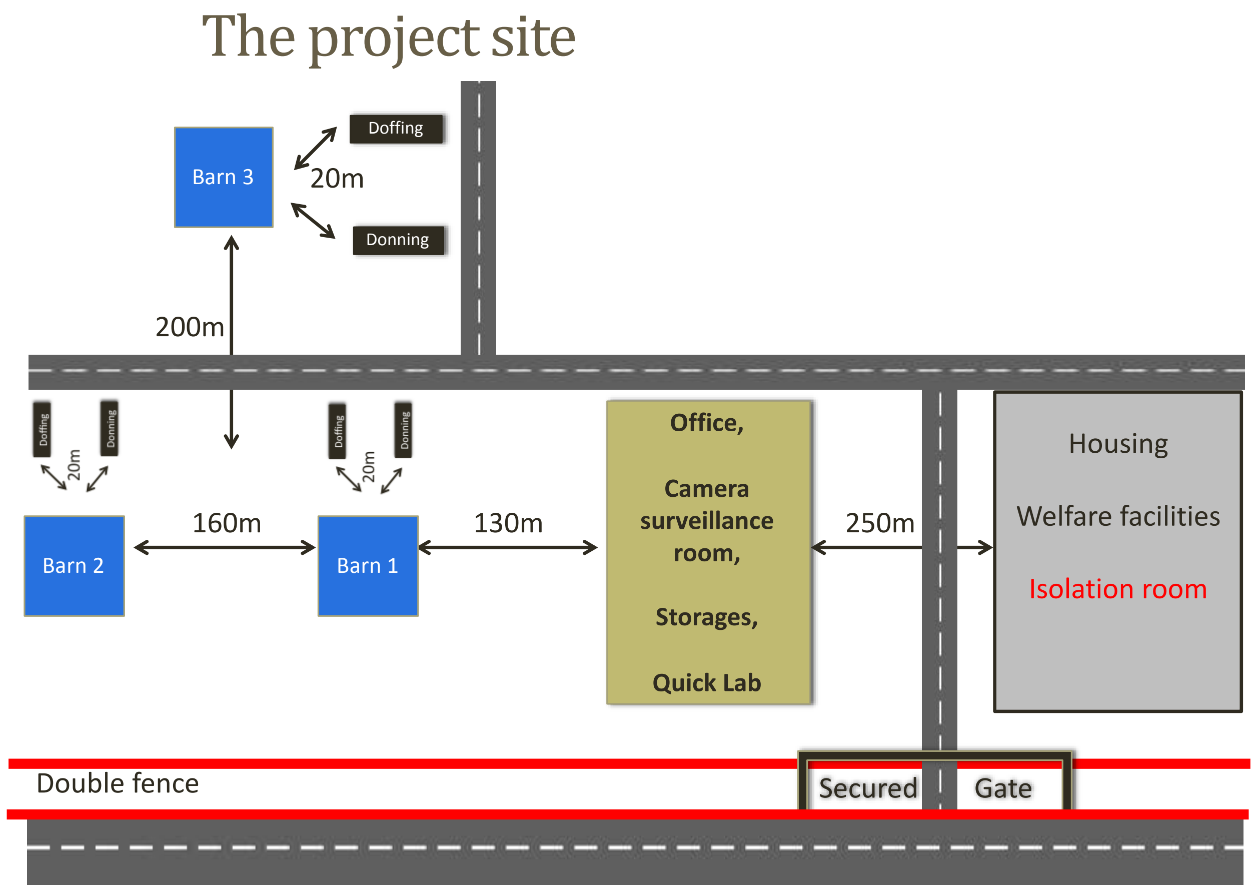
Seronegative dromedary calves, below 1 year old, were immunised with PBS (n=2) or ChAdOx1-eGFP (n=1) in group AG01 (black triangles) or with ChAdOx1 MERS in group AG02 (n=3; blue squares). Older calves, below 2 years old, were immunised with ChAdOx1 MERS in group AG03 (n=3; red circles). Anti-spike antibody levels were assessed in endpoint titre ELISA **(A)** and Euroimmun ELISA kits **(B)**. Correlation between values in both assays is presented in (**C**). Linear regression between the antibody titres in the Endpoint ELISA and Euroimmun ELISA ratios showed a significant relationship with $R^2 = 0.82$ and $p < 0.0001$; and a significant correlation with Pearson $r = 0.90$ and $p < 0.0001$.

II. Supplementary Photographs

Photographs taken during the course of the study (*Reference to this article must be made when citing or sharing these photos*).



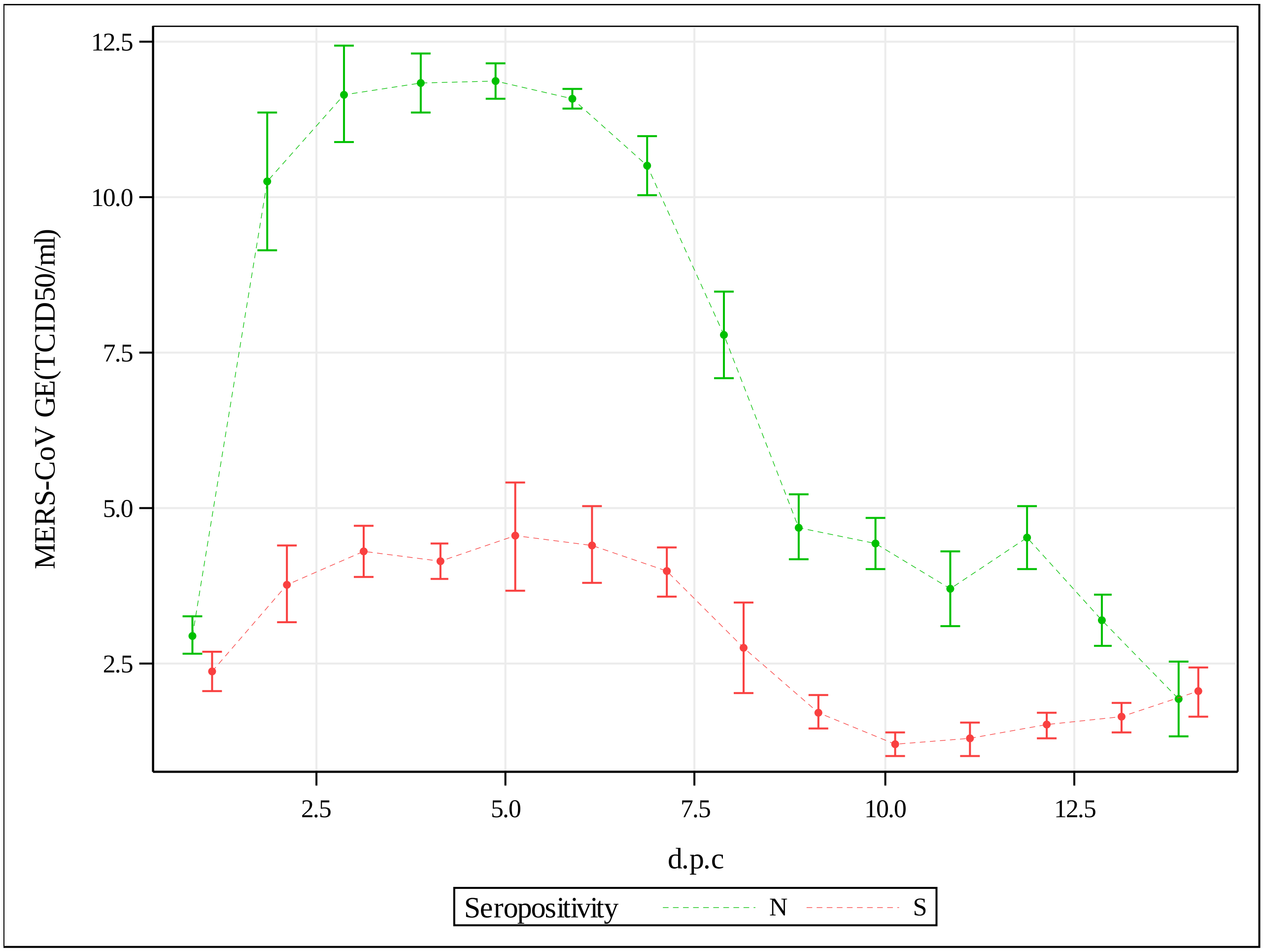
III. Supplementary diagram on the layout of the research farm

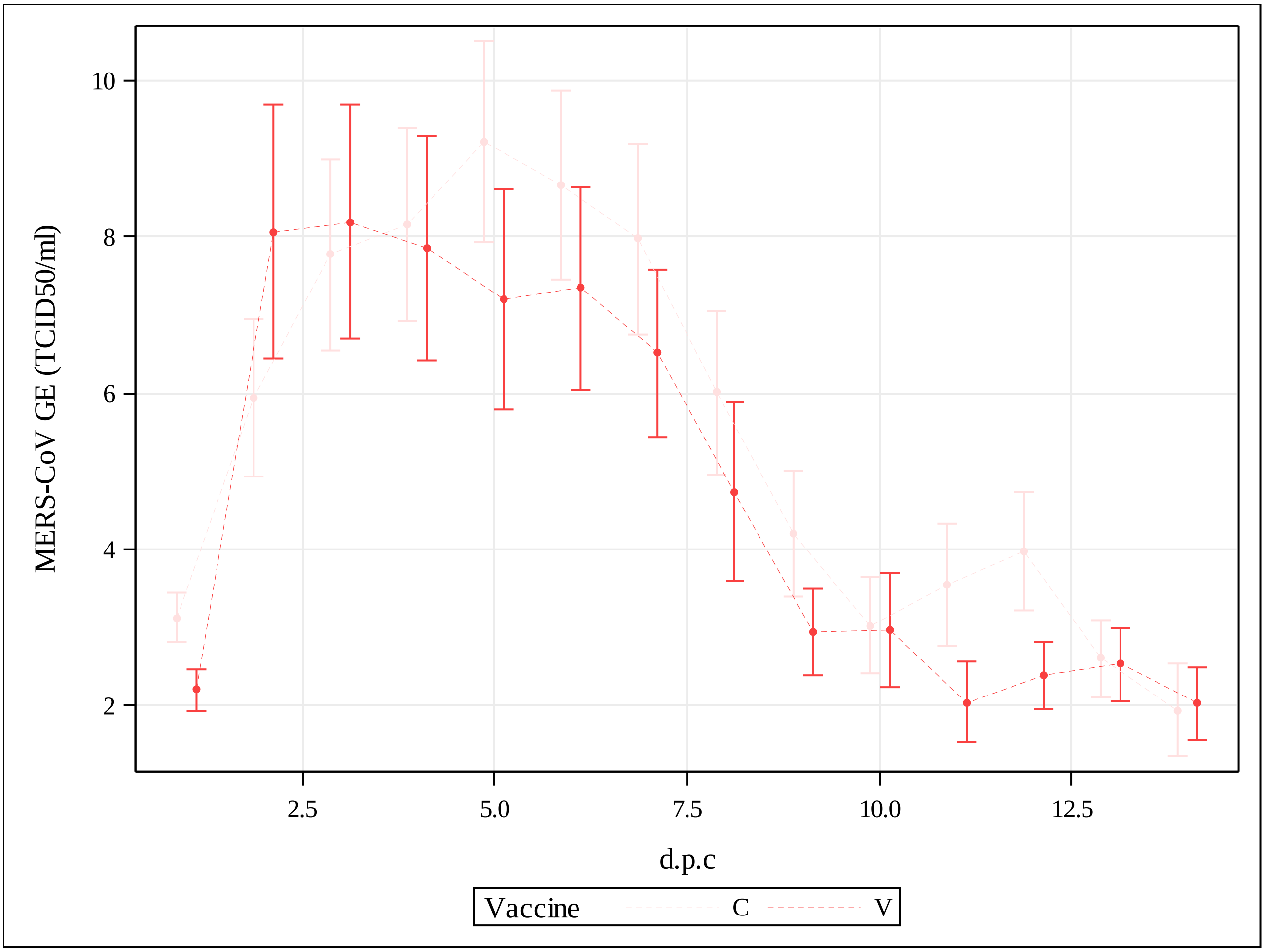


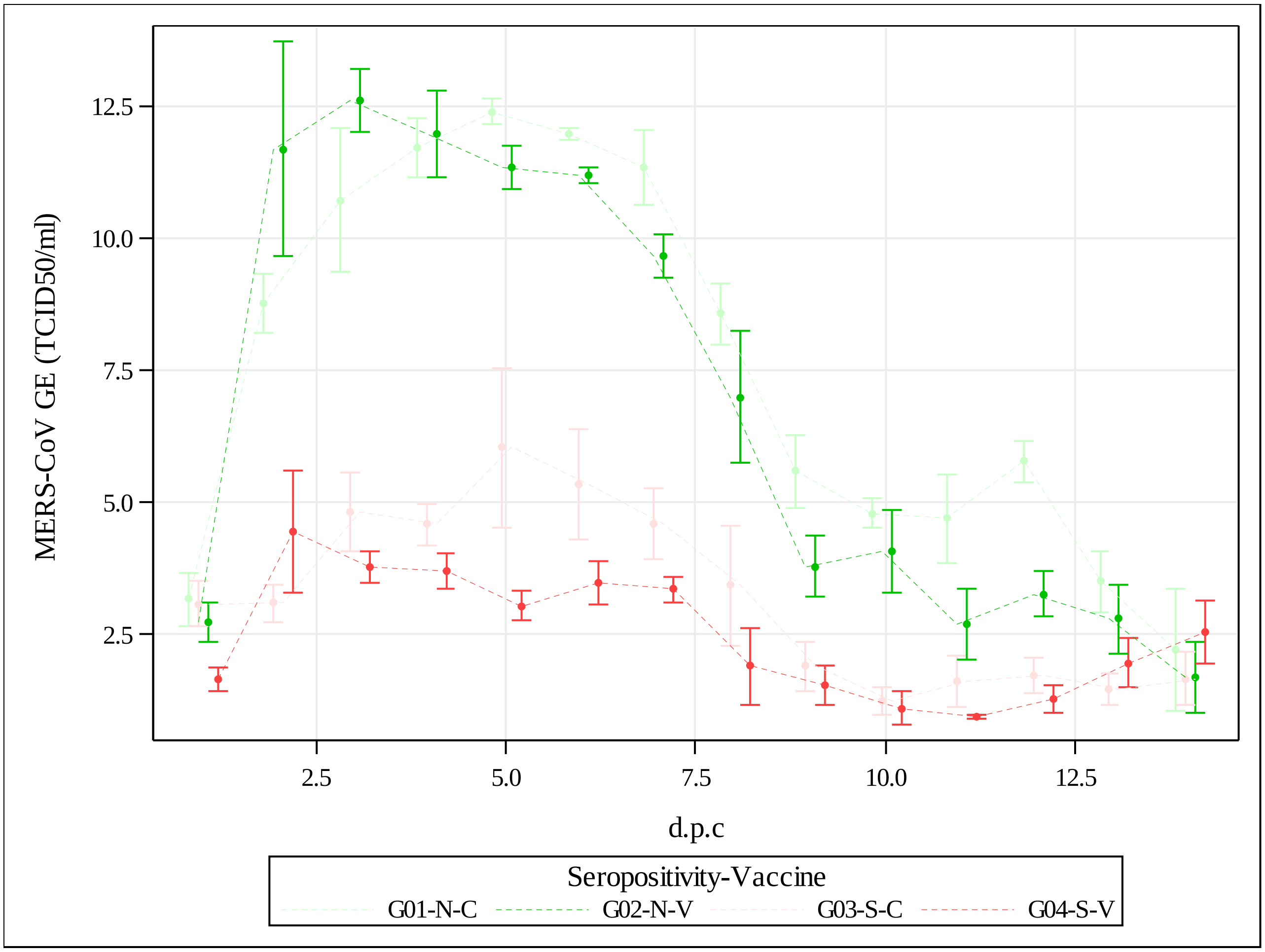
Distance is shown in metre (m). Each barn has donning and doffing dedicated areas

VI. Supplementary details on the statistical analysis

Note: Groups: -V (also called G01-N-V); -C (also called G02-N-C); +V (also called G03-S-V); +C (also called G04-S-C) where C: control, V: vaccinated, S: seropositive, N:naïve (seronegative).




MERS-CoV GE (TCID50/ml)
10
8
6
4
2
2.5
5.0
7.5
10.0
12.5
d.p.c
Vaccine
C
V


MERS-CoV GE (TCID50/ml)
12.5
10.0
7.5
5.0
2.5
d.p.c
2.5
5.0
7.5
10.0
12.5
Seropositivity-Vaccine
G01-N-C
G02-N-V
G03-S-C
G04-S-V

The Mixed Procedure

Model Information	
Data Set	WORK.DATA0
Dependent Variable	Virus_titre_post_challenge
Covariance Structure	Autoregressive
Subject Effect	CamelID
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Between-Within

Class Level Information		
Class	**Levels**	**Values**
d_p_c_	14	1 2 3 4 5 6 7 8 9 10 11 12 13 14
CamelID	20	11 12 13 14 15 21 22 23 24 25 31 32 33 34 35 41 42 43 44 45
Seropositivity	2	0 1
Vaccine	2	0 1

Dimensions	
Covariance Parameters	2
Columns in X	75
Columns in Z	0
Subjects	20
Max Obs Per Subject	14

Number of Observations	
Number of Observations Read	280
Number of Observations Used	258
Number of Observations Not Used	22

Iteration History			
Iteration	**Evaluations**	**-2 Res Log Like**	**Criterion**
0	1	888.78662828	
1	2	865.57005966	0.00000020
2	1	865.57001358	0.00000000

The Mixed Procedure

Convergence criteria met.

Estimated R Matrix for CamelID 11

Row	Col1	Col2	Col3	Col4	Col5	Col6	Col7	Col8	Col9	Col10	Col11	Col12	Col13	Col14
1	2.4075	0.7709	0.2468	0.07904	0.02531	0.008104	0.002595	0.000831	0.000266	0.000085	0.000027	8.736E-6	2.797E-6	8.957E-7
2	0.7709	2.4075	0.7709	0.2468	0.07904	0.02531	0.008104	0.002595	0.000831	0.000266	0.000085	0.000027	8.736E-6	2.797E-6
3	0.2468	0.7709	2.4075	0.7709	0.2468	0.07904	0.02531	0.008104	0.002595	0.000831	0.000266	0.000085	0.000027	8.736E-6
4	0.07904	0.2468	0.7709	2.4075	0.7709	0.2468	0.07904	0.02531	0.008104	0.002595	0.000831	0.000266	0.000085	0.000027
5	0.02531	0.07904	0.2468	0.7709	2.4075	0.7709	0.2468	0.07904	0.02531	0.008104	0.002595	0.000831	0.000266	0.000085
6	0.008104	0.02531	0.07904	0.2468	0.7709	2.4075	0.7709	0.2468	0.07904	0.02531	0.008104	0.002595	0.000831	0.000266
7	0.002595	0.008104	0.02531	0.07904	0.2468	0.7709	2.4075	0.7709	0.2468	0.07904	0.02531	0.008104	0.002595	0.000831
8	0.000831	0.002595	0.008104	0.02531	0.07904	0.2468	0.7709	2.4075	0.7709	0.2468	0.07904	0.02531	0.008104	0.002595
9	0.000266	0.000831	0.002595	0.008104	0.02531	0.07904	0.2468	0.7709	2.4075	0.7709	0.2468	0.07904	0.02531	0.008104
10	0.000085	0.000266	0.000831	0.002595	0.008104	0.02531	0.07904	0.2468	0.7709	2.4075	0.7709	0.2468	0.07904	0.02531
11	0.000027	0.000085	0.000266	0.000831	0.002595	0.008104	0.02531	0.07904	0.2468	0.7709	2.4075	0.7709	0.2468	0.07904
12	8.736E-6	0.000027	0.000085	0.000266	0.000831	0.002595	0.008104	0.02531	0.07904	0.2468	0.7709	2.4075	0.7709	0.2468
13	2.797E-6	8.736E-6	0.000027	0.000085	0.000266	0.000831	0.002595	0.008104	0.02531	0.07904	0.2468	0.7709	2.4075	0.7709
14	8.957E-7	2.797E-6	8.736E-6	0.000027	0.000085	0.000266	0.000831	0.002595	0.008104	0.02531	0.07904	0.2468	0.7709	2.4075

Estimated R Correlation Matrix for CamelID 11

Row	Col1	Col2	Col3	Col4	Col5	Col6	Col7	Col8	Col9	Col10	Col11	Col12	Col13	Col14
1	1.0000	0.3202	0.1025	0.03283	0.01051	0.003366	0.001078	0.000345	0.000111	0.000035	0.000011	3.629E-6	1.162E-6	3.721E-7
2	0.3202	1.0000	0.3202	0.1025	0.03283	0.01051	0.003366	0.001078	0.000345	0.000111	0.000035	0.000011	3.629E-6	1.162E-6
3	0.1025	0.3202	1.0000	0.3202	0.1025	0.03283	0.01051	0.003366	0.001078	0.000345	0.000111	0.000035	0.000011	3.629E-6
4	0.03283	0.1025	0.3202	1.0000	0.3202	0.1025	0.03283	0.01051	0.003366	0.001078	0.000345	0.000111	0.000035	0.000011
5	0.01051	0.03283	0.1025	0.3202	1.0000	0.3202	0.1025	0.03283	0.01051	0.003366	0.001078	0.000345	0.000111	0.000035
6	0.003366	0.01051	0.03283	0.1025	0.3202	1.0000	0.3202	0.1025	0.03283	0.01051	0.003366	0.001078	0.000345	0.000111
7	0.001078	0.003366	0.01051	0.03283	0.1025	0.3202	1.0000	0.3202	0.1025	0.03283	0.01051	0.003366	0.001078	0.000345
8	0.000345	0.001078	0.003366	0.01051	0.03283	0.1025	0.3202	1.0000	0.3202	0.1025	0.03283	0.01051	0.003366	0.001078
9	0.000111	0.000345	0.001078	0.003366	0.01051	0.03283	0.1025	0.3202	1.0000	0.3202	0.1025	0.03283	0.01051	0.003366
10	0.000035	0.000111	0.000345	0.001078	0.003366	0.01051	0.03283	0.1025	0.3202	1.0000	0.3202	0.1025	0.03283	0.01051
11	0.000011	0.000035	0.000111	0.000345	0.001078	0.003366	0.01051	0.03283	0.1025	0.3202	1.0000	0.3202	0.1025	0.03283
12	3.629E-6	0.000011	0.000035	0.000111	0.000345	0.001078	0.003366	0.01051	0.03283	0.1025	0.3202	1.0000	0.3202	0.1025
13	1.162E-6	3.629E-6	0.000011	0.000035	0.000111	0.000345	0.001078	0.003366	0.01051	0.03283	0.1025	0.3202	1.0000	0.3202
14	3.721E-7	1.162E-6	3.629E-6	0.000011	0.000035	0.000111	0.000345	0.001078	0.003366	0.01051	0.03283	0.1025	0.3202	1.0000

The Mixed Procedure

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
AR(1)	CamelID	0.3202
Residual		2.4075

Fit Statistics	
-2 Res Log Likelihood	865.6
AIC (smaller is better)	869.6
AICC (smaller is better)	869.6
BIC (smaller is better)	871.6

Null Model Likelihood Ratio Test		
DF	**Chi-Square**	**Pr > ChiSq**
1	23.22	<.0001

Solution for Fixed Effects								
Effect	**d_p_c_**	**Seropositivity**	**Vaccine**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Intercept				2.1258	0.7302	17	2.91	0.0097
d_p_c_	1			-0.2160	0.9457	199	-0.23	0.8195
d_p_c_	2			2.7107	0.9457	199	2.87	0.0046
d_p_c_	3			2.3857	0.9457	199	2.52	0.0124
d_p_c_	4			1.8748	0.9457	199	1.98	0.0488
d_p_c_	5			1.4098	0.9457	199	1.49	0.1376
d_p_c_	6			1.6282	0.9456	199	1.72	0.0867
d_p_c_	7			1.1233	0.9455	199	1.19	0.2363
d_p_c_	8			-0.1722	0.9725	199	-0.18	0.8597
d_p_c_	9			-0.9122	1.0268	199	-0.89	0.3754
d_p_c_	10			-1.1342	1.0120	199	-1.12	0.2638
d_p_c_	11			-1.5910	1.0177	199	-1.56	0.1196
d_p_c_	12			-1.4066	0.9349	199	-1.50	0.1340
d_p_c_	13			-0.4489	0.9779	199	-0.46	0.6467
d_p_c_	14			0	.	.	.	.
Seropositivity		0		-0.1757	0.7818	17	-0.22	0.8248

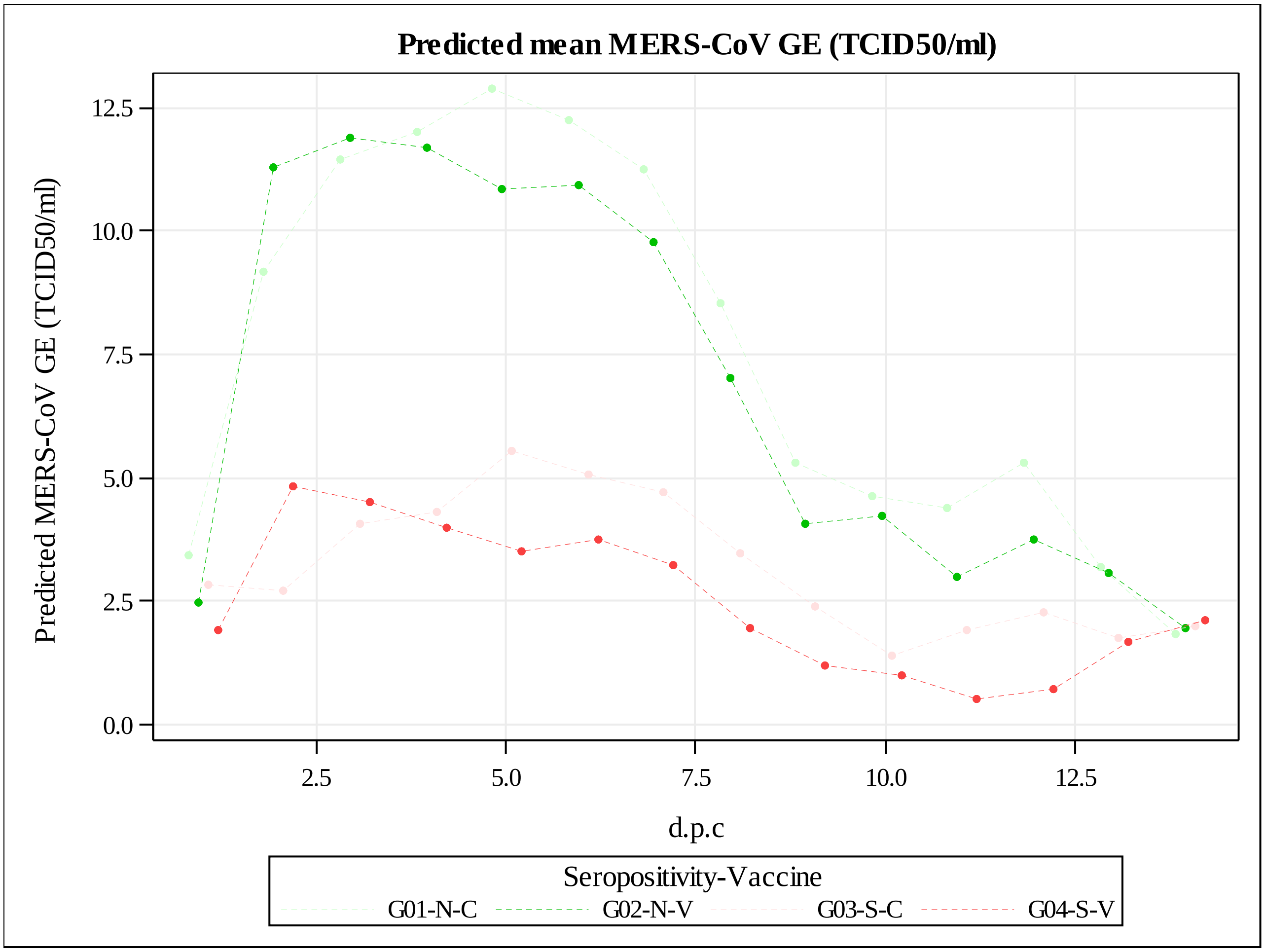
The Mixed Procedure

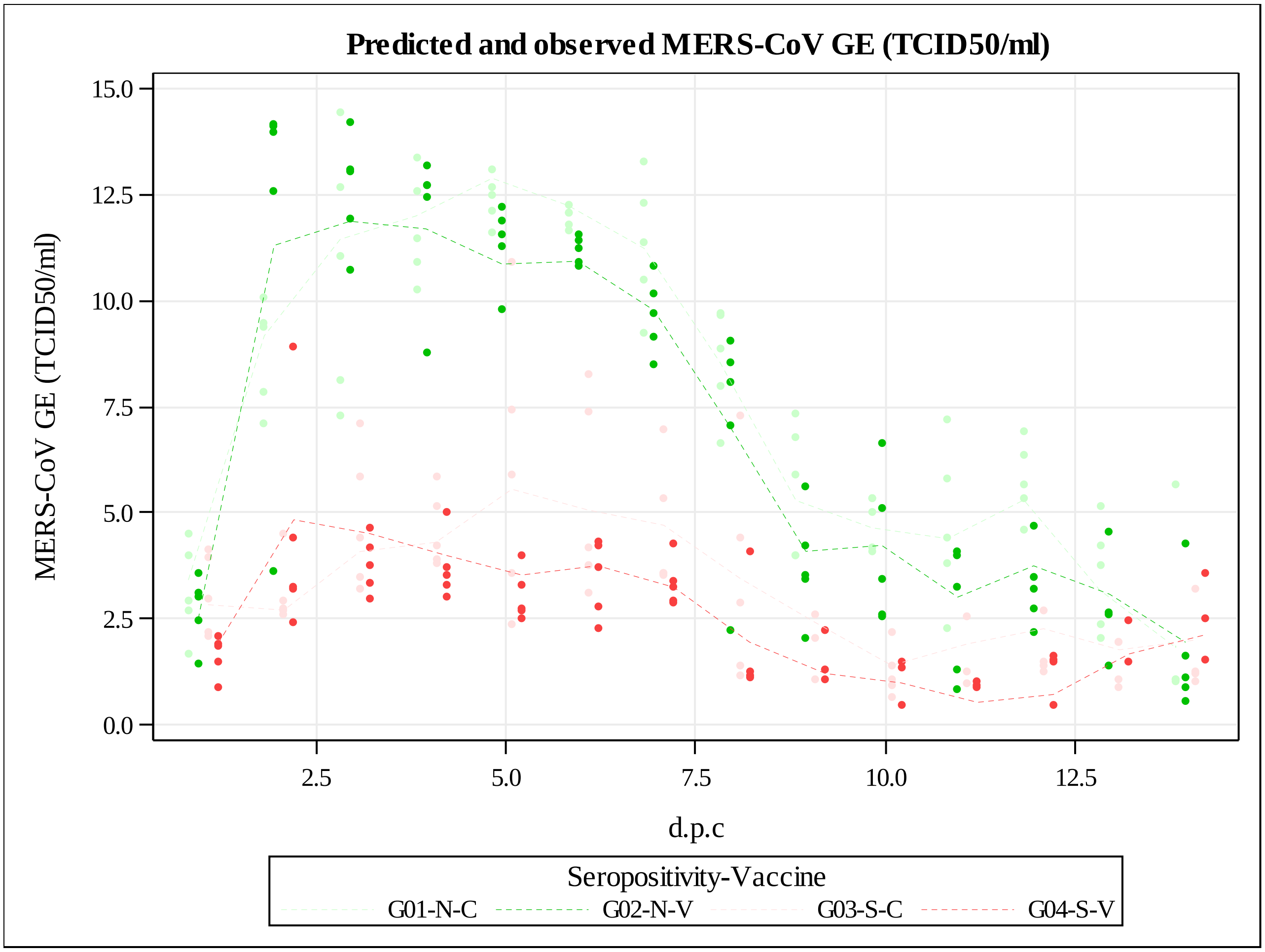
Solution for Fixed Effects								
Effect	**d_p_c_**	**Seropositivity**	**Vaccine**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Seropositivity		1		0	.	.	.	.
Vaccine			0	-0.1233	0.7757	17	-0.16	0.8755
Vaccine			1	0	.	.	.	.
d_p_c_*Seropositivit	1	0		0.7593	1.0454	199	0.73	0.4685
d_p_c_*Seropositivit	1	1		0	.	.	.	.
d_p_c_*Seropositivit	2	0		6.6415	1.0454	199	6.35	<.0001
d_p_c_*Seropositivit	2	1		0	.	.	.	.
d_p_c_*Seropositivit	3	0		7.5349	1.0453	199	7.21	<.0001
d_p_c_*Seropositivit	3	1		0	.	.	.	.
d_p_c_*Seropositivit	4	0		7.8667	1.0453	199	7.53	<.0001
d_p_c_*Seropositivit	4	1		0	.	.	.	.
d_p_c_*Seropositivit	5	0		7.5032	1.0453	199	7.18	<.0001
d_p_c_*Seropositivit	5	1		0	.	.	.	.
d_p_c_*Seropositivit	6	0		7.3501	1.0453	199	7.03	<.0001
d_p_c_*Seropositivit	6	1		0	.	.	.	.
d_p_c_*Seropositivit	7	0		6.7013	1.0452	199	6.41	<.0001
d_p_c_*Seropositivit	7	1		0	.	.	.	.
d_p_c_*Seropositivit	8	0		5.2587	1.0559	199	4.98	<.0001
d_p_c_*Seropositivit	8	1		0	.	.	.	.
d_p_c_*Seropositivit	9	0		3.0555	1.1063	199	2.76	0.0063
d_p_c_*Seropositivit	9	1		0	.	.	.	.
d_p_c_*Seropositivit	10	0		3.4176	1.0695	199	3.20	0.0016
d_p_c_*Seropositivit	10	1		0	.	.	.	.
d_p_c_*Seropositivit	11	0		2.6550	1.0945	199	2.43	0.0162
d_p_c_*Seropositivit	11	1		0	.	.	.	.
d_p_c_*Seropositivit	12	0		3.2084	1.0200	199	3.15	0.0019
d_p_c_*Seropositivit	12	1		0	.	.	.	.
d_p_c_*Seropositivit	13	0		1.5815	0.9793	199	1.61	0.1079
d_p_c_*Seropositivit	13	1		0	.	.	.	.
d_p_c_*Seropositivit	14	0		0	.	.	.	.
d_p_c_*Seropositivit	14	1		0	.	.	.	.
d_p_c_*Vaccine	1		0	1.0482	1.0407	199	1.01	0.3151
d_p_c_*Vaccine	1		1	0	.	.	.	.
d_p_c_*Vaccine	2		0	-2.0051	1.0407	199	-1.93	0.0554

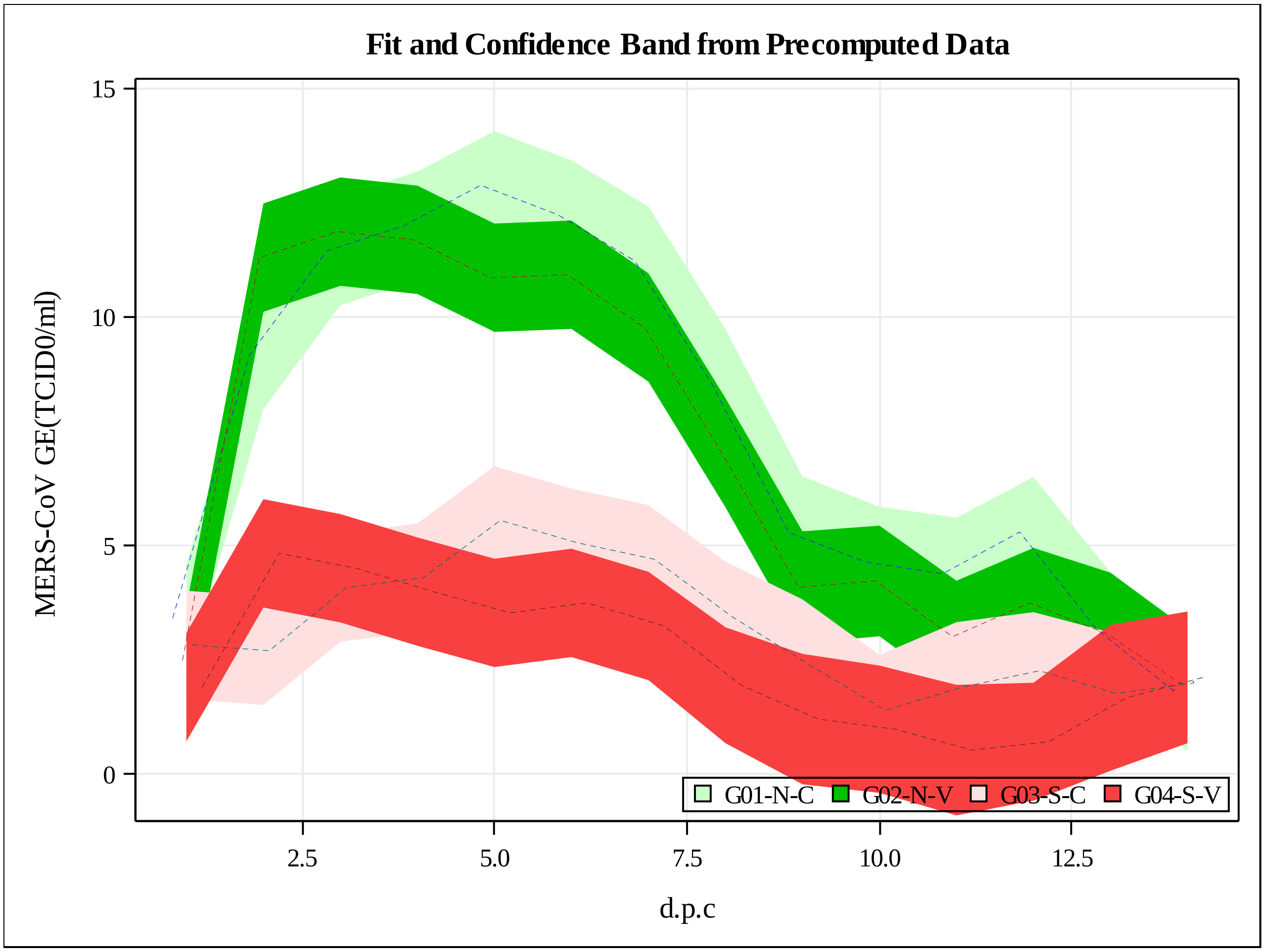
The Mixed Procedure

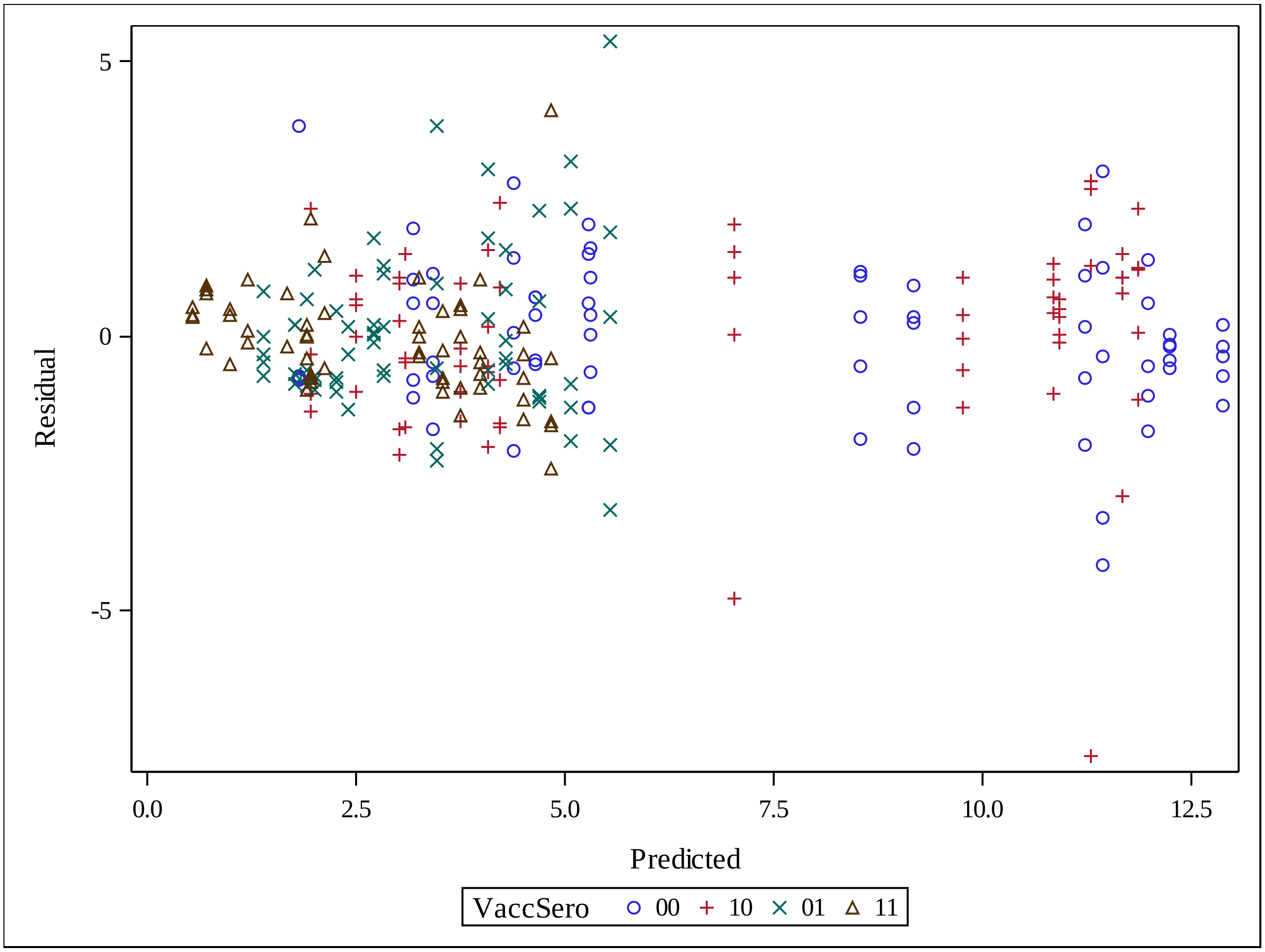
Solution for Fixed Effects								
Effect	**d_p_c_**	**Seropositivity**	**Vaccine**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
d_p_c_*Vaccine	2		1	0	.	.	.	.
d_p_c_*Vaccine	3		0	-0.3004	1.0407	199	-0.29	0.7732
d_p_c_*Vaccine	3		1	0	.	.	.	.
d_p_c_*Vaccine	4		0	0.4296	1.0407	199	0.41	0.6802
d_p_c_*Vaccine	4		1	0	.	.	.	.
d_p_c_*Vaccine	5		0	2.1447	1.0407	199	2.06	0.0406
d_p_c_*Vaccine	5		1	0	.	.	.	.
d_p_c_*Vaccine	6		0	1.4380	1.0407	199	1.38	0.1686
d_p_c_*Vaccine	6		1	0	.	.	.	.
d_p_c_*Vaccine	7		0	1.5824	1.0406	199	1.52	0.1299
d_p_c_*Vaccine	7		1	0	.	.	.	.
d_p_c_*Vaccine	8		0	1.6318	1.0514	199	1.55	0.1222
d_p_c_*Vaccine	8		1	0	.	.	.	.
d_p_c_*Vaccine	9		0	1.3215	1.0865	199	1.22	0.2253
d_p_c_*Vaccine	9		1	0	.	.	.	.
d_p_c_*Vaccine	10		0	0.5375	1.0649	199	0.50	0.6143
d_p_c_*Vaccine	10		1	0	.	.	.	.
d_p_c_*Vaccine	11		0	1.5011	1.0736	199	1.40	0.1636
d_p_c_*Vaccine	11		1	0	.	.	.	.
d_p_c_*Vaccine	12		0	1.6740	1.0126	199	1.65	0.0999
d_p_c_*Vaccine	12		1	0	.	.	.	.
d_p_c_*Vaccine	13		0	0.2197	0.9688	199	0.23	0.8208
d_p_c_*Vaccine	13		1	0	.	.	.	.
d_p_c_*Vaccine	14		0	0	.	.	.	.
d_p_c_*Vaccine	14		1	0	.	.	.	.

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
d_p_c_	13	199	35.21	<.0001
Seropositivity	1	17	284.28	<.0001
Vaccine	1	17	6.89	0.0177
d_p_c_*Seropositivit	13	199	11.44	<.0001
d_p_c_*Vaccine	13	199	2.36	0.0059


Predicted mean MERS-CoV GE (TCID50/ml)
Predicted MERS-CoV GE (TCID50/ml)
0.0
2.5
5.0
7.5
10.0
12.5
2.5
5.0
7.5
10.0
12.5
d.p.c
Seropositivity-Vaccine
G01-N-C
G02-N-V
G03-S-C
G04-S-V


Predicted and observed MERS-CoV GE (TCID50/ml)
MERS-CoV GE (TCID50/ml)
0.0
2.5
5.0
7.5
10.0
12.5
15.0
2.5
5.0
7.5
10.0
12.5
d.p.c
Seropositivity-Vaccine
G01-N-C
G02-N-V
G03-S-C
G04-S-V

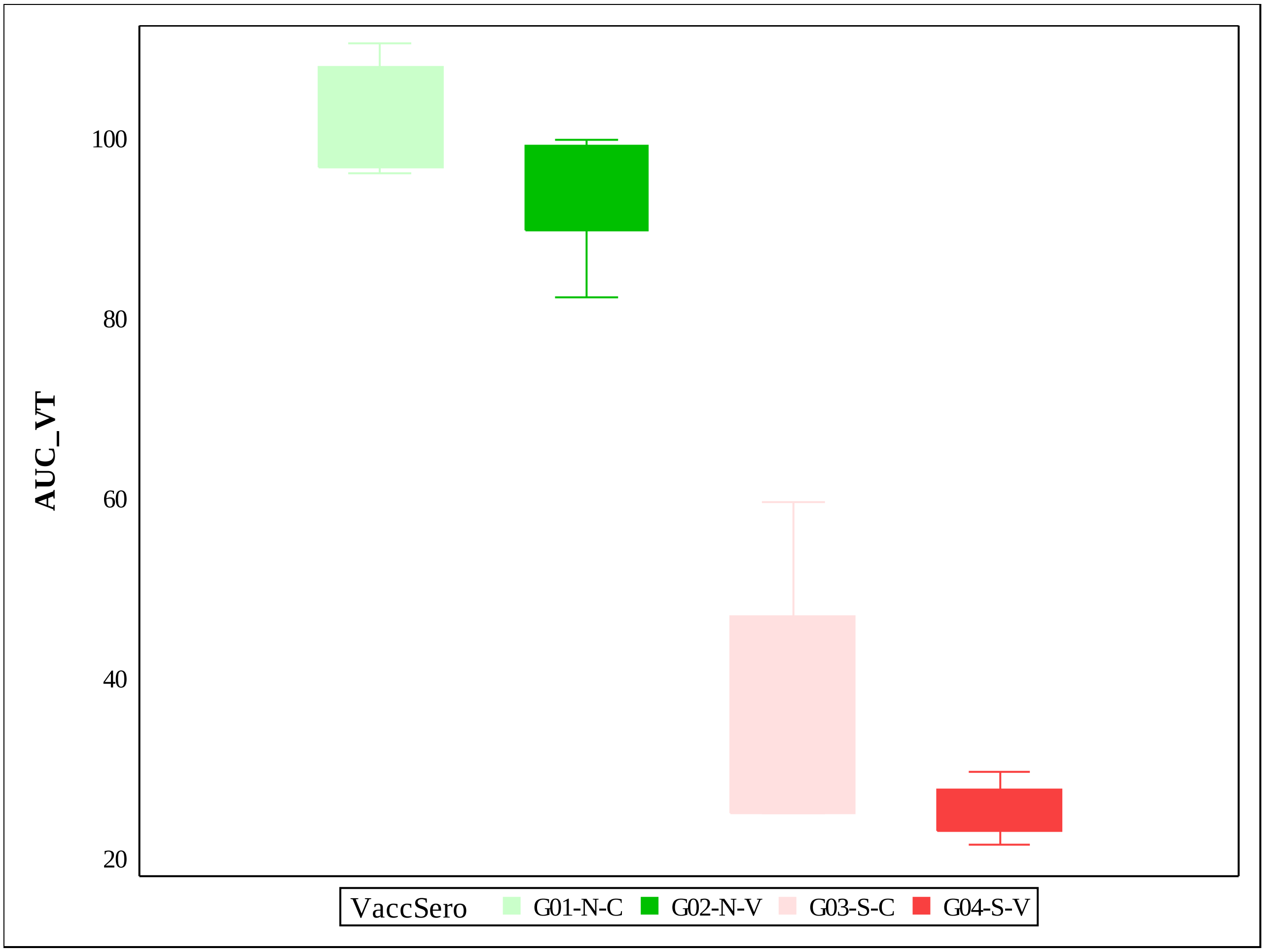

Fit and Confidence Band from Precomputed Data
MERS-CoV GE(TCID0/ml)
15
10
5
0
2.5
5.0
7.5
10.0
12.5
d.p.c
G01-N-C
G02-N-V
G03-S-C
G04-S-V

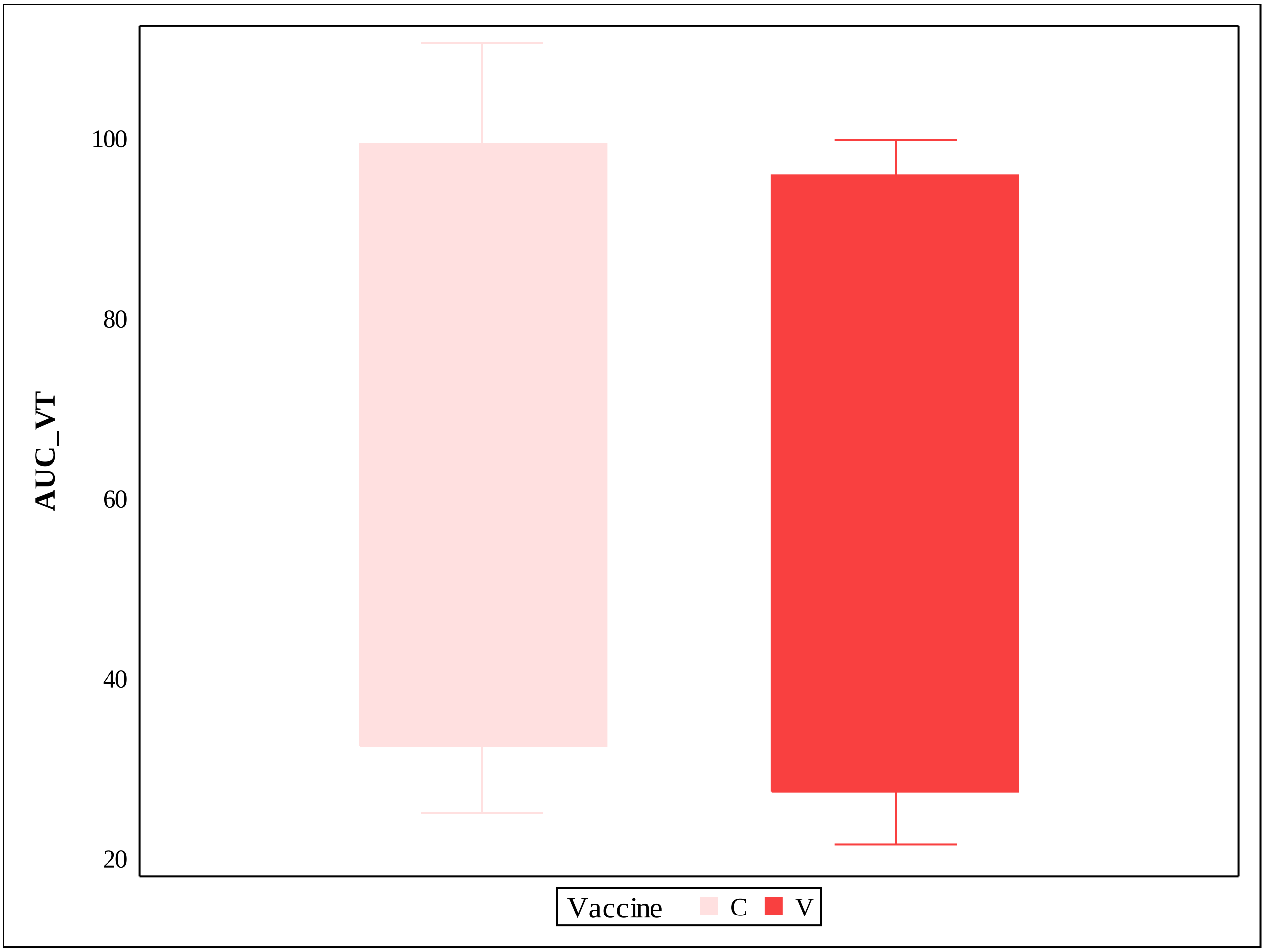


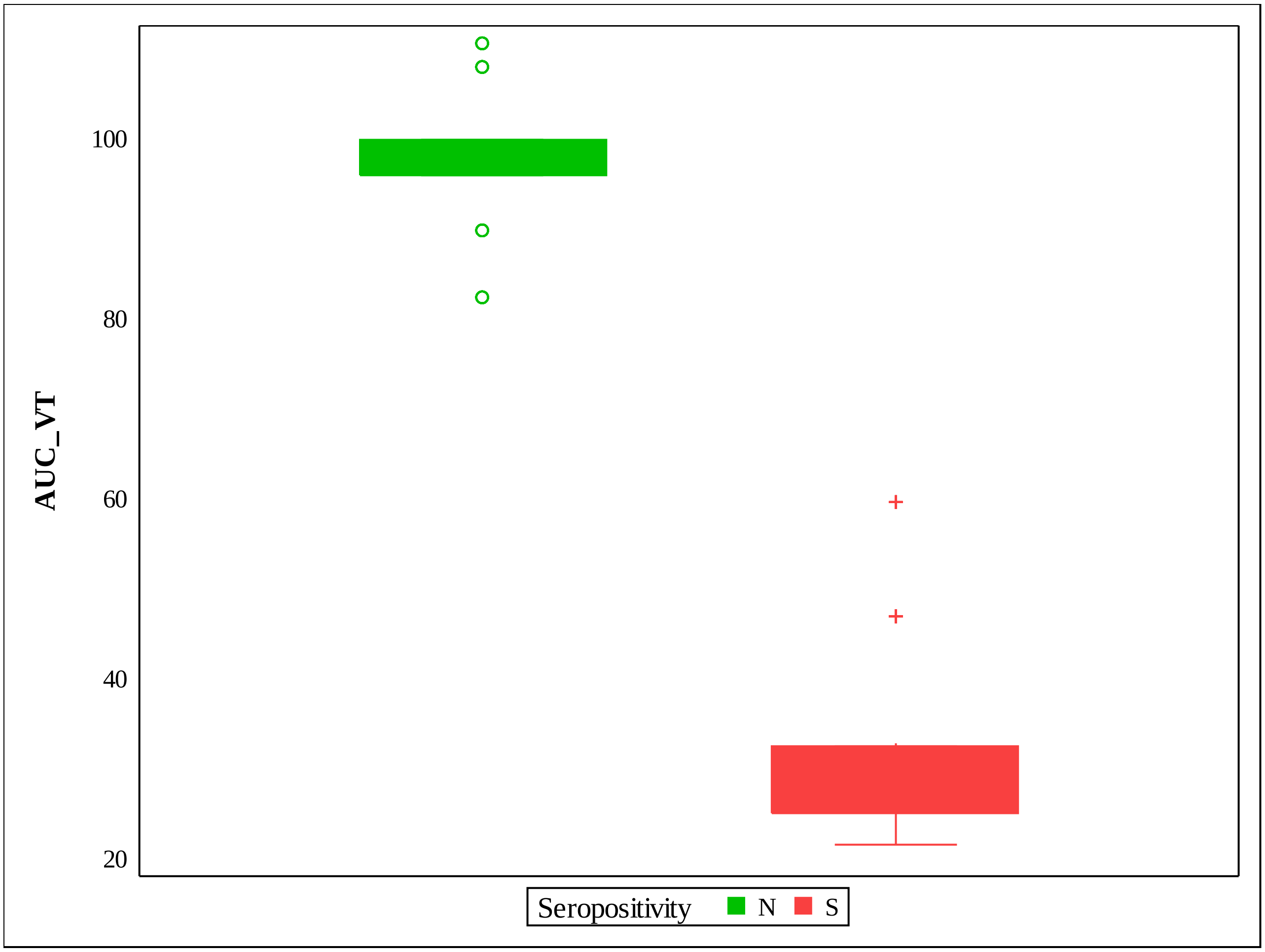
5
0
-5
Residual
0.0
2.5
5.0
7.5
10.0
12.5
Predicted
VaccSero
○ 00
+ 10
× 01
△ 11

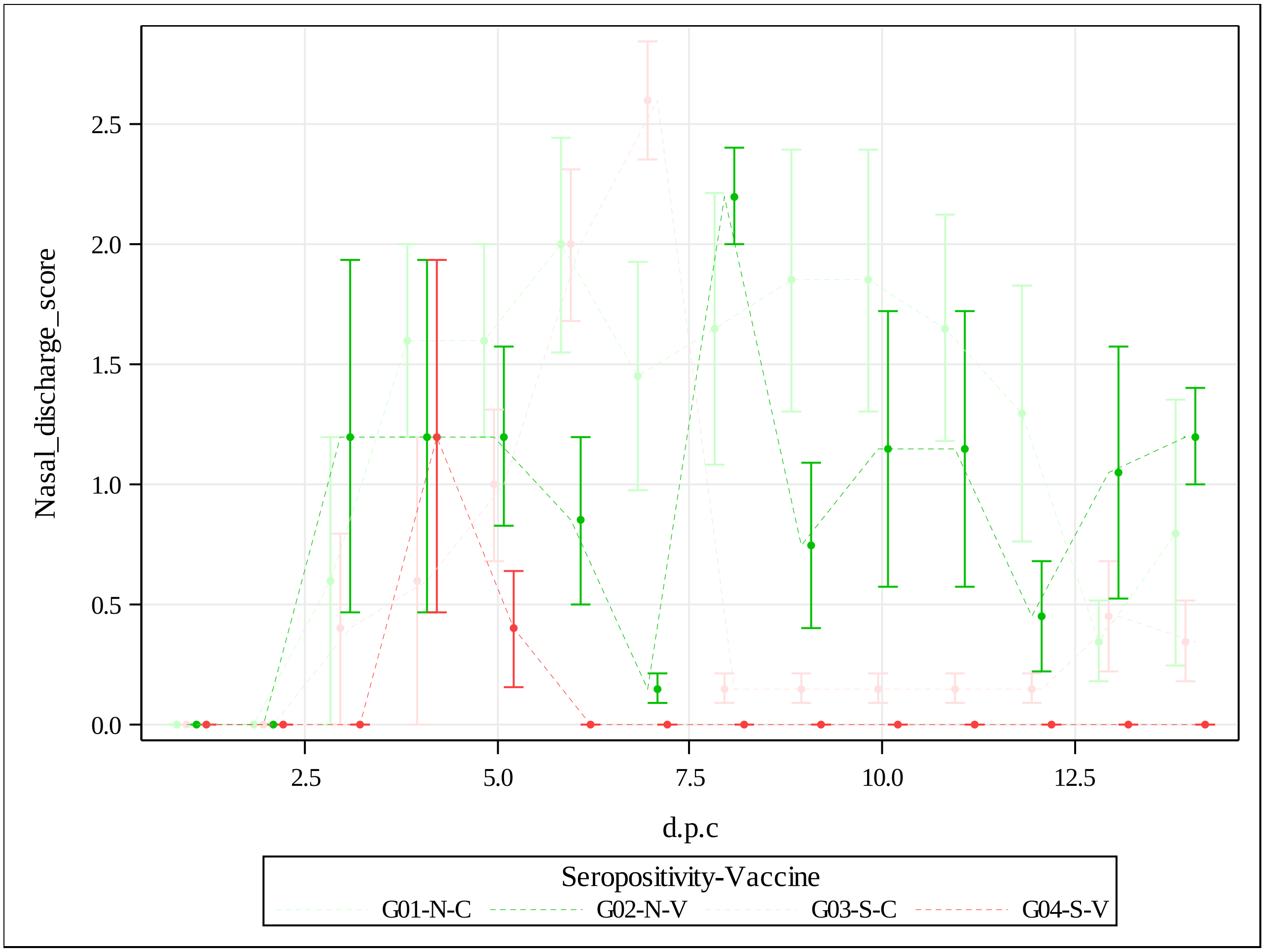
The MEANS Procedure

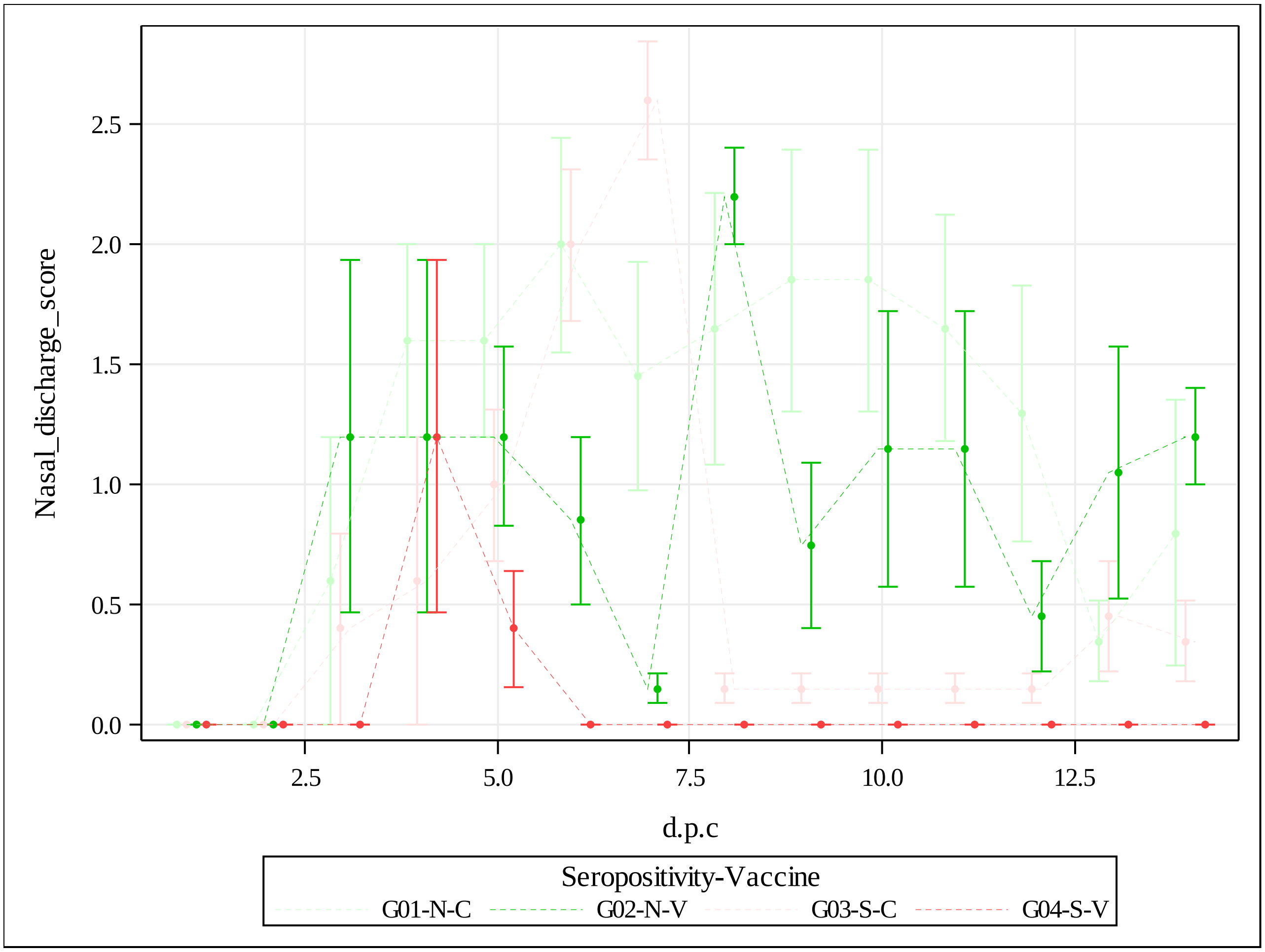
Analysis Variable : areaRect		
CamelID	**N Obs**	**Sum**
11	14	96.7460828
12	14	107.8487485
13	14	96.1011404
14	14	110.5697307
15	14	99.4420902
21	14	99.9070033
22	14	89.6964427
23	14	95.9023348
24	14	99.2099861
25	14	82.3156968
31	14	59.5459676
32	14	24.9573527
33	14	32.5058721
34	14	25.1188792
35	14	46.9222652
41	14	27.4368385
42	14	23.0595128
43	14	27.6492982
44	14	21.6483356
45	14	29.6137791


AUC_VT
100
80
60
40
20
VaccSero
G01-N-C
G02-N-V
G03-S-C
G04-S-V


AUC_VT
100
80
60
40
20
Vaccine
C
V


AUC_VT
100
80
60
40
20
Seropositivity
🟩 N
🟥 S


Nasal_discharge_score
0.0
0.5
1.0
1.5
2.0
2.5
2.5
5.0
7.5
10.0
12.5
d.p.c
Seropositivity-Vaccine
G01-N-C
G02-N-V
G03-S-C
G04-S-V


Nasal_discharge_score
0.0
0.5
1.0
1.5
2.0
2.5
2.5
5.0
7.5
10.0
12.5
d.p.c
Seropositivity-Vaccine
G01-N-C
G02-N-V
G03-S-C
G04-S-V

The Mixed Procedure

Model Information

Data Set	WORK.DATAN0
Dependent Variable	Nasal_discharge_score
Covariance Structure	Autoregressive
Subject Effect	CamelID
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Between-Within

Class Level Information

Class	Levels	Values
d_p_c_	14	1 2 3 4 5 6 7 8 9 10 11 12 13 14
CamelID	20	11 12 13 14 15 21 22 23 24 25 31 32 33 34 35 41 42 43 44 45
Seropositivity	2	0 1
Vaccine	2	0 1

Dimensions

Covariance Parameters	2
Columns in X	75
Columns in Z	0
Subjects	20
Max Obs Per Subject	14

Number of Observations

Number of Observations Read	280
Number of Observations Used	280
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	649.84733028	
1	2	569.06863698	0.00000262
2	1	569.06846431	0.00000000

The Mixed Procedure

Convergence criteria met.

Estimated R Matrix for CamelID 11

Row	Col1	Col2	Col3	Col4	Col5	Col6	Col7	Col8	Col9	Col10	Col11	Col12
1	0.6028	0.3195	0.1694	0.08978	0.04759	0.02522	0.01337	0.007087	0.003756	0.001991	0.001055	0.000559
2	0.3195	0.6028	0.3195	0.1694	0.08978	0.04759	0.02522	0.01337	0.007087	0.003756	0.001991	0.001055
3	0.1694	0.3195	0.6028	0.3195	0.1694	0.08978	0.04759	0.02522	0.01337	0.007087	0.003756	0.001991
4	0.08978	0.1694	0.3195	0.6028	0.3195	0.1694	0.08978	0.04759	0.02522	0.01337	0.007087	0.003756
5	0.04759	0.08978	0.1694	0.3195	0.6028	0.3195	0.1694	0.08978	0.04759	0.02522	0.01337	0.007087
6	0.02522	0.04759	0.08978	0.1694	0.3195	0.6028	0.3195	0.1694	0.08978	0.04759	0.02522	0.01337
7	0.01337	0.02522	0.04759	0.08978	0.1694	0.3195	0.6028	0.3195	0.1694	0.08978	0.04759	0.02522
8	0.007087	0.01337	0.02522	0.04759	0.08978	0.1694	0.3195	0.6028	0.3195	0.1694	0.08978	0.04759
9	0.003756	0.007087	0.01337	0.02522	0.04759	0.08978	0.1694	0.3195	0.6028	0.3195	0.1694	0.08978
10	0.001991	0.003756	0.007087	0.01337	0.02522	0.04759	0.08978	0.1694	0.3195	0.6028	0.3195	0.1694
11	0.001055	0.001991	0.003756	0.007087	0.01337	0.02522	0.04759	0.08978	0.1694	0.3195	0.6028	0.3195
12	0.000559	0.001055	0.001991	0.003756	0.007087	0.01337	0.02522	0.04759	0.08978	0.1694	0.3195	0.6028
13	0.000297	0.000559	0.001055	0.001991	0.003756	0.007087	0.01337	0.02522	0.04759	0.08978	0.1694	0.3195
14	0.000157	0.000297	0.000559	0.001055	0.001991	0.003756	0.007087	0.01337	0.02522	0.04759	0.08978	0.1694

Estimated R Matrix for CamelID 11

Row	Col13	Col14
1	0.000297	0.000157
2	0.000559	0.000297
3	0.001055	0.000559
4	0.001991	0.001055
5	0.003756	0.001991
6	0.007087	0.003756
7	0.01337	0.007087
8	0.02522	0.01337
9	0.04759	0.02522
10	0.08978	0.04759
11	0.1694	0.08978
12	0.3195	0.1694
13	0.6028	0.3195
14	0.3195	0.6028

The Mixed Procedure

Estimated R Correlation Matrix for CamelID 11												
Row	**Col1**	**Col2**	**Col3**	**Col4**	**Col5**	**Col6**	**Col7**	**Col8**	**Col9**	**Col10**	**Col11**	**Col12**
1	1.0000	0.5301	0.2810	0.1489	0.07894	0.04184	0.02218	0.01176	0.006231	0.003303	0.001751	0.000928
2	0.5301	1.0000	0.5301	0.2810	0.1489	0.07894	0.04184	0.02218	0.01176	0.006231	0.003303	0.001751
3	0.2810	0.5301	1.0000	0.5301	0.2810	0.1489	0.07894	0.04184	0.02218	0.01176	0.006231	0.003303
4	0.1489	0.2810	0.5301	1.0000	0.5301	0.2810	0.1489	0.07894	0.04184	0.02218	0.01176	0.006231
5	0.07894	0.1489	0.2810	0.5301	1.0000	0.5301	0.2810	0.1489	0.07894	0.04184	0.02218	0.01176
6	0.04184	0.07894	0.1489	0.2810	0.5301	1.0000	0.5301	0.2810	0.1489	0.07894	0.04184	0.02218
7	0.02218	0.04184	0.07894	0.1489	0.2810	0.5301	1.0000	0.5301	0.2810	0.1489	0.07894	0.04184
8	0.01176	0.02218	0.04184	0.07894	0.1489	0.2810	0.5301	1.0000	0.5301	0.2810	0.1489	0.07894
9	0.006231	0.01176	0.02218	0.04184	0.07894	0.1489	0.2810	0.5301	1.0000	0.5301	0.2810	0.1489
10	0.003303	0.006231	0.01176	0.02218	0.04184	0.07894	0.1489	0.2810	0.5301	1.0000	0.5301	0.2810
11	0.001751	0.003303	0.006231	0.01176	0.02218	0.04184	0.07894	0.1489	0.2810	0.5301	1.0000	0.5301
12	0.000928	0.001751	0.003303	0.006231	0.01176	0.02218	0.04184	0.07894	0.1489	0.2810	0.5301	1.0000
13	0.000492	0.000928	0.001751	0.003303	0.006231	0.01176	0.02218	0.04184	0.07894	0.1489	0.2810	0.5301
14	0.000261	0.000492	0.000928	0.001751	0.003303	0.006231	0.01176	0.02218	0.04184	0.07894	0.1489	0.2810

Estimated R Correlation Matrix for CamelID 11		
Row	**Col13**	**Col14**
1	0.000492	0.000261
2	0.000928	0.000492
3	0.001751	0.000928
4	0.003303	0.001751
5	0.006231	0.003303
6	0.01176	0.006231
7	0.02218	0.01176
8	0.04184	0.02218
9	0.07894	0.04184
10	0.1489	0.07894
11	0.2810	0.1489
12	0.5301	0.2810
13	1.0000	0.5301
14	0.5301	1.0000

The Mixed Procedure

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
AR(1)	CamelID	0.5301
Residual		0.6028

Fit Statistics	
-2 Res Log Likelihood	569.1
AIC (smaller is better)	573.1
AICC (smaller is better)	573.1
BIC (smaller is better)	575.1

Null Model Likelihood Ratio Test		
DF	**Chi-Square**	**Pr > ChiSq**
1	80.78	<.0001

Solution for Fixed Effects								
Effect	**d_p_c_**	**Seropositivity**	**Vaccine**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Intercept				0.1875	0.3007	17	0.62	0.5412
d_p_c_	1			-0.1875	0.4252	221	-0.44	0.6597
d_p_c_	2			-0.1875	0.4252	221	-0.44	0.6596
d_p_c_	3			0.06250	0.4251	221	0.15	0.8832
d_p_c_	4			0.7625	0.4249	221	1.79	0.0741
d_p_c_	5			0.2625	0.4246	221	0.62	0.5370
d_p_c_	6			0.02500	0.4239	221	0.06	0.9530
d_p_c_	7			0.1375	0.4228	221	0.33	0.7453
d_p_c_	8			-0.01250	0.4205	221	-0.03	0.9763
d_p_c_	9			-0.4250	0.4163	221	-1.02	0.3084
d_p_c_	10			-0.3250	0.4081	221	-0.80	0.4267
d_p_c_	11			-0.2750	0.3923	221	-0.70	0.4841
d_p_c_	12			-0.3625	0.3606	221	-1.01	0.3159
d_p_c_	13			0.1000	0.2915	221	0.34	0.7319
d_p_c_	14			0	.	.	.	.
Seropositivity		0		0.8250	0.3472	17	2.38	0.0295

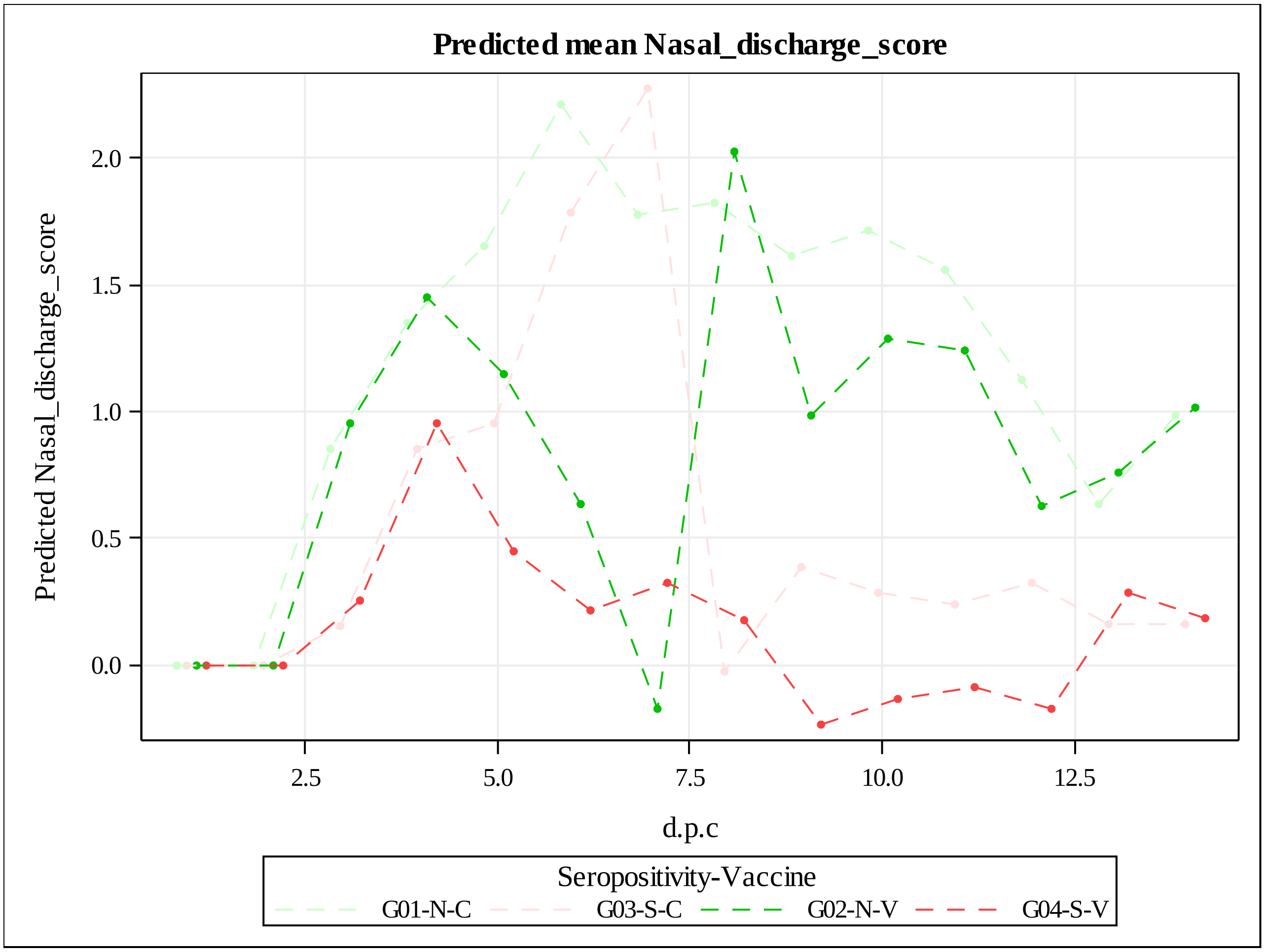
The Mixed Procedure

Solution for Fixed Effects								
Effect	**d_p_c_**	**Seropositivity**	**Vaccine**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Seropositivity		1		0	.	.	.	.
Vaccine			0	-0.02500	0.3472	17	-0.07	0.9434
Vaccine			1	0	.	.	.	.
d_p_c_*Seropositivit	1	0		-0.8250	0.4910	221	-1.68	0.0943
d_p_c_*Seropositivit	1	1		0	.	.	.	.
d_p_c_*Seropositivit	2	0		-0.8250	0.4909	221	-1.68	0.0943
d_p_c_*Seropositivit	2	1		0	.	.	.	.
d_p_c_*Seropositivit	3	0		-0.1250	0.4908	221	-0.25	0.7992
d_p_c_*Seropositivit	3	1		0	.	.	.	.
d_p_c_*Seropositivit	4	0		-0.3250	0.4906	221	-0.66	0.5084
d_p_c_*Seropositivit	4	1		0	.	.	.	.
d_p_c_*Seropositivit	5	0		-0.1250	0.4902	221	-0.25	0.7990
d_p_c_*Seropositivit	5	1		0	.	.	.	.
d_p_c_*Seropositivit	6	0		-0.4000	0.4895	221	-0.82	0.4147
d_p_c_*Seropositivit	6	1		0	.	.	.	.
d_p_c_*Seropositivit	7	0		-1.3250	0.4882	221	-2.71	0.0072
d_p_c_*Seropositivit	7	1		0	.	.	.	.
d_p_c_*Seropositivit	8	0		1.0250	0.4856	221	2.11	0.0359
d_p_c_*Seropositivit	8	1		0	.	.	.	.
d_p_c_*Seropositivit	9	0		0.4000	0.4807	221	0.83	0.4062
d_p_c_*Seropositivit	9	1		0	.	.	.	.
d_p_c_*Seropositivit	10	0		0.6000	0.4713	221	1.27	0.2043
d_p_c_*Seropositivit	10	1		0	.	.	.	.
d_p_c_*Seropositivit	11	0		0.5000	0.4530	221	1.10	0.2709
d_p_c_*Seropositivit	11	1		0	.	.	.	.
d_p_c_*Seropositivit	12	0		-0.02500	0.4164	221	-0.06	0.9522
d_p_c_*Seropositivit	12	1		0	.	.	.	.
d_p_c_*Seropositivit	13	0		-0.3500	0.3366	221	-1.04	0.2996
d_p_c_*Seropositivit	13	1		0	.	.	.	.
d_p_c_*Seropositivit	14	0		0	.	.	.	.
d_p_c_*Seropositivit	14	1		0	.	.	.	.
d_p_c_*Vaccine	1		0	0.02500	0.4910	221	0.05	0.9594
d_p_c_*Vaccine	1		1	0	.	.	.	.
d_p_c_*Vaccine	2		0	0.02500	0.4909	221	0.05	0.9594

The Mixed Procedure

Solution for Fixed Effects								
Effect	**d_p_c_**	**Seropositivity**	**Vaccine**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
d_p_c_*Vaccine	2		1	0	.	.	.	.
d_p_c_*Vaccine	3		0	-0.07500	0.4908	221	-0.15	0.8787
d_p_c_*Vaccine	3		1	0	.	.	.	.
d_p_c_*Vaccine	4		0	-0.07500	0.4906	221	-0.15	0.8786
d_p_c_*Vaccine	4		1	0	.	.	.	.
d_p_c_*Vaccine	5		0	0.5250	0.4902	221	1.07	0.2854
d_p_c_*Vaccine	5		1	0	.	.	.	.
d_p_c_*Vaccine	6		0	1.6000	0.4895	221	3.27	0.0013
d_p_c_*Vaccine	6		1	0	.	.	.	.
d_p_c_*Vaccine	7		0	1.9750	0.4882	221	4.05	<.0001
d_p_c_*Vaccine	7		1	0	.	.	.	.
d_p_c_*Vaccine	8		0	-0.1750	0.4856	221	-0.36	0.7189
d_p_c_*Vaccine	8		1	0	.	.	.	.
d_p_c_*Vaccine	9		0	0.6500	0.4807	221	1.35	0.1777
d_p_c_*Vaccine	9		1	0	.	.	.	.
d_p_c_*Vaccine	10		0	0.4500	0.4713	221	0.95	0.3407
d_p_c_*Vaccine	10		1	0	.	.	.	.
d_p_c_*Vaccine	11		0	0.3500	0.4530	221	0.77	0.4406
d_p_c_*Vaccine	11		1	0	.	.	.	.
d_p_c_*Vaccine	12		0	0.5250	0.4164	221	1.26	0.2087
d_p_c_*Vaccine	12		1	0	.	.	.	.
d_p_c_*Vaccine	13		0	-0.1000	0.3366	221	-0.30	0.7667
d_p_c_*Vaccine	13		1	0	.	.	.	.
d_p_c_*Vaccine	14		0	0	.	.	.	.
d_p_c_*Vaccine	14		1	0	.	.	.	.

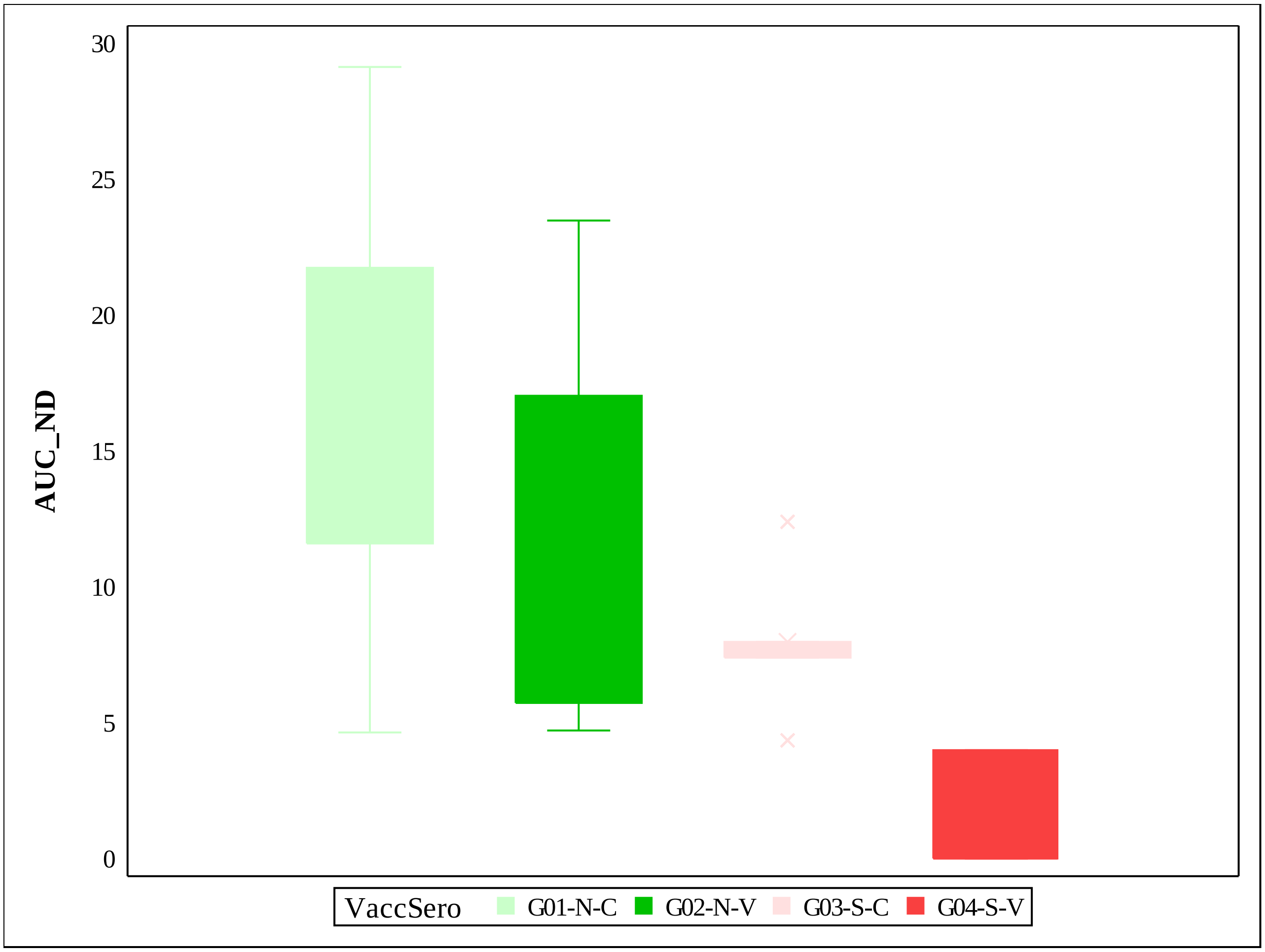
Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
d_p_c_	13	221	3.65	<.0001
Seropositivity	1	17	19.33	0.0004
Vaccine	1	17	5.82	0.0274
d_p_c_*Seropositivit	13	221	4.86	<.0001
d_p_c_*Vaccine	13	221	4.64	<.0001

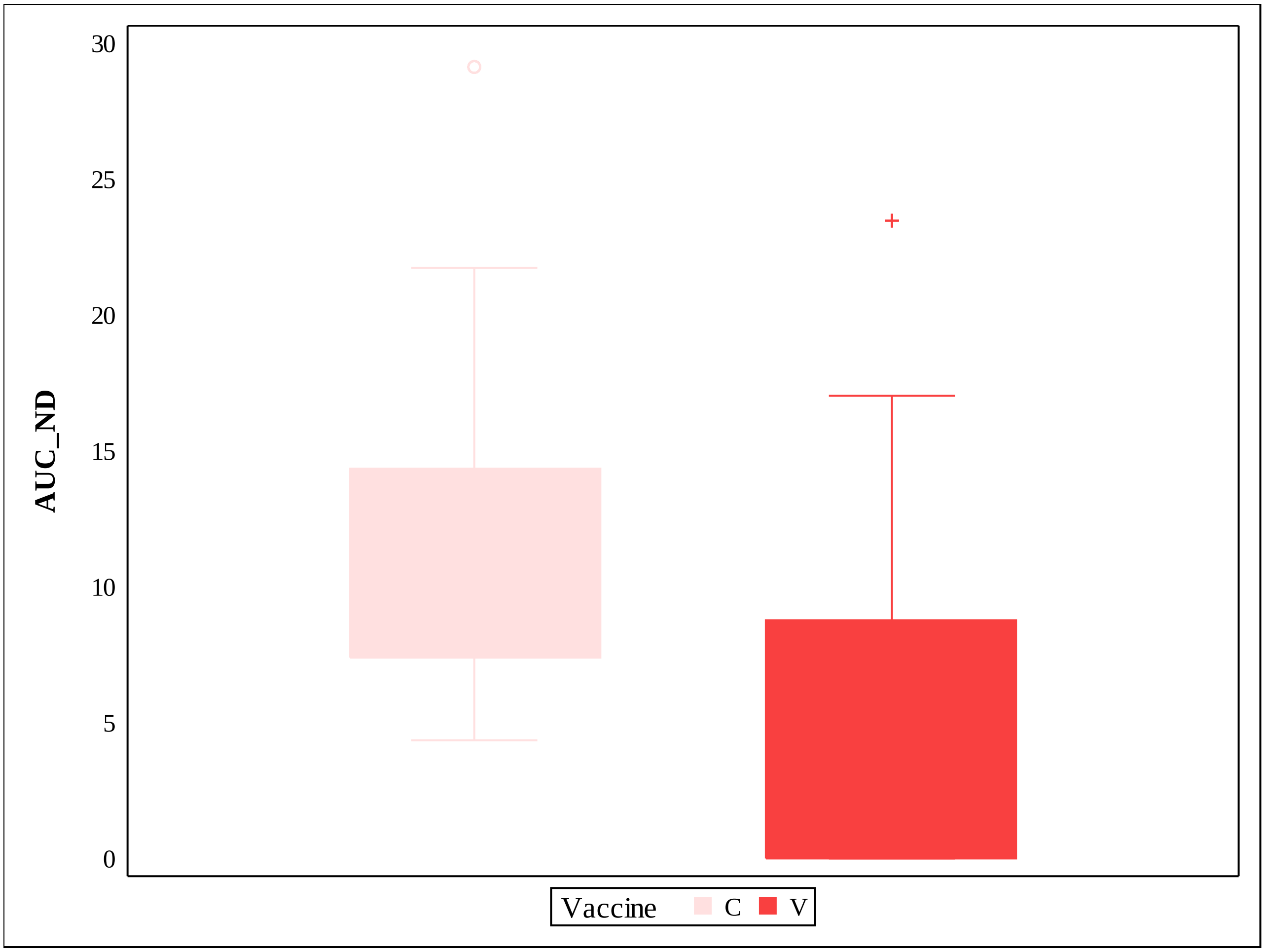

Predicted mean Nasal_discharge_score
Predicted Nasal_discharge_score
2.0
1.5
1.0
0.5
0.0
2.5
5.0
7.5
10.0
12.5
d.p.c
Seropositivity-Vaccine
G01-N-C
G03-S-C
G02-N-V
G04-S-V

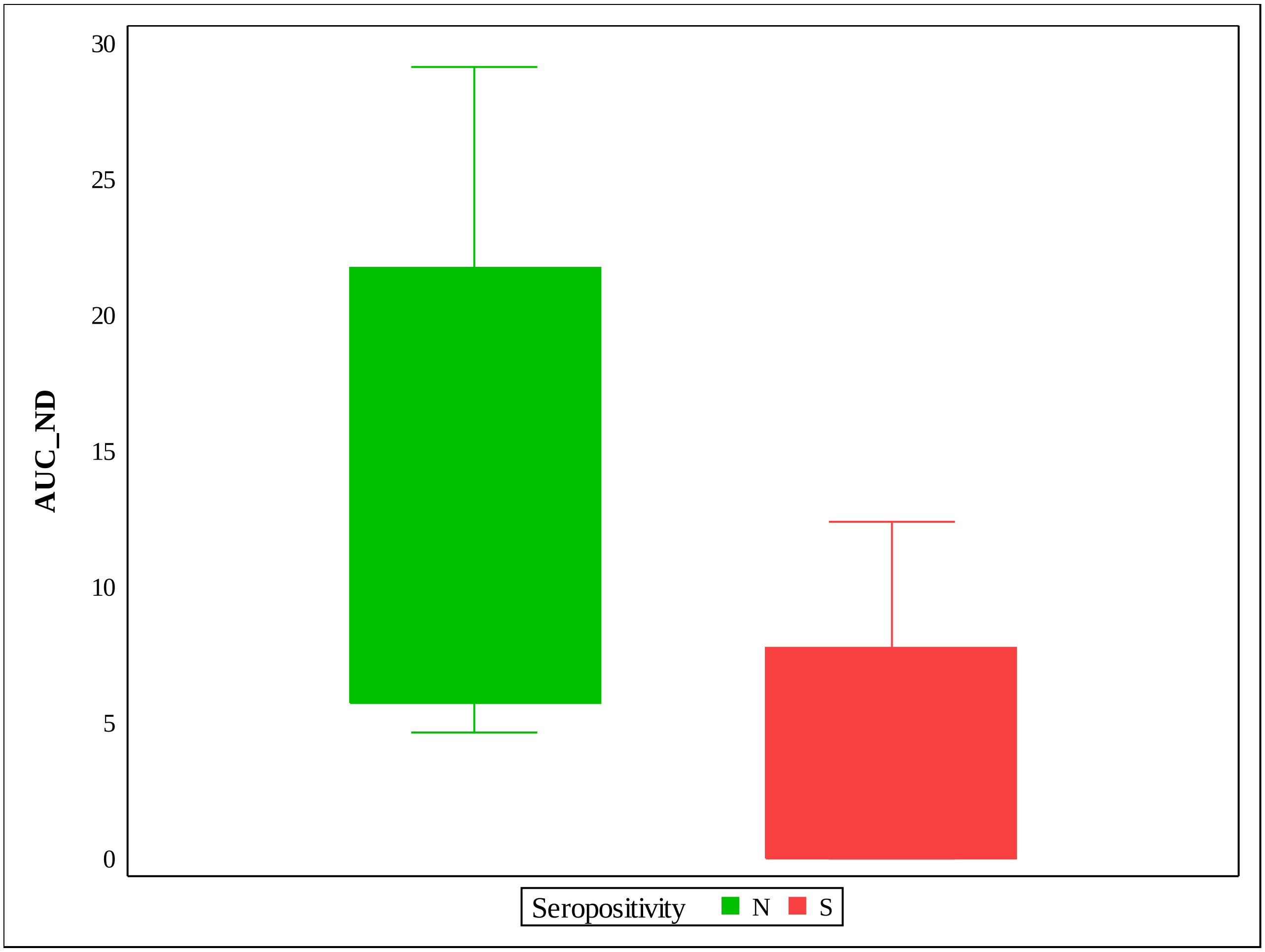
Predicted mean Nasal_discharge_score

The MEANS Procedure

Analysis Variable : areaRect		
CamelID	**N Obs**	**Sum**
11	14	11.6250000
12	14	14.3750000
13	14	29.1250000
14	14	21.7500000
15	14	4.6250000
21	14	8.7500000
22	14	23.5000000
23	14	17.0000000
24	14	4.7500000
25	14	5.7500000
31	14	4.3750000
32	14	8.0000000
33	14	7.7500000
34	14	12.3750000
35	14	7.3750000
41	14	0
42	14	0
43	14	4.0000000
44	14	0
45	14	4.0000000


30
25
20
15
10
5
0
AUC_ND
VaccSero
G01-N-C
G02-N-V
G03-S-C
G04-S-V


AUC_ND
30
25
20
15
10
5
0
Vaccine
C
V


30
25
20
15
10
5
0
AUC_ND
Seropositivity
N
S

The GLM Procedure

Class Level Information		
Class	**Levels**	**Values**
Seropositivity	2	N S
Vaccine	2	C V

Number of Observations Read	20
Number of Observations Used	20

The GLM Procedure

Dependent Variable: AUC_VT

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	3	22279.06791	7426.35597	87.97	<.0001
Error	16	1350.65958	84.41622		
Corrected Total	19	23629.72749			

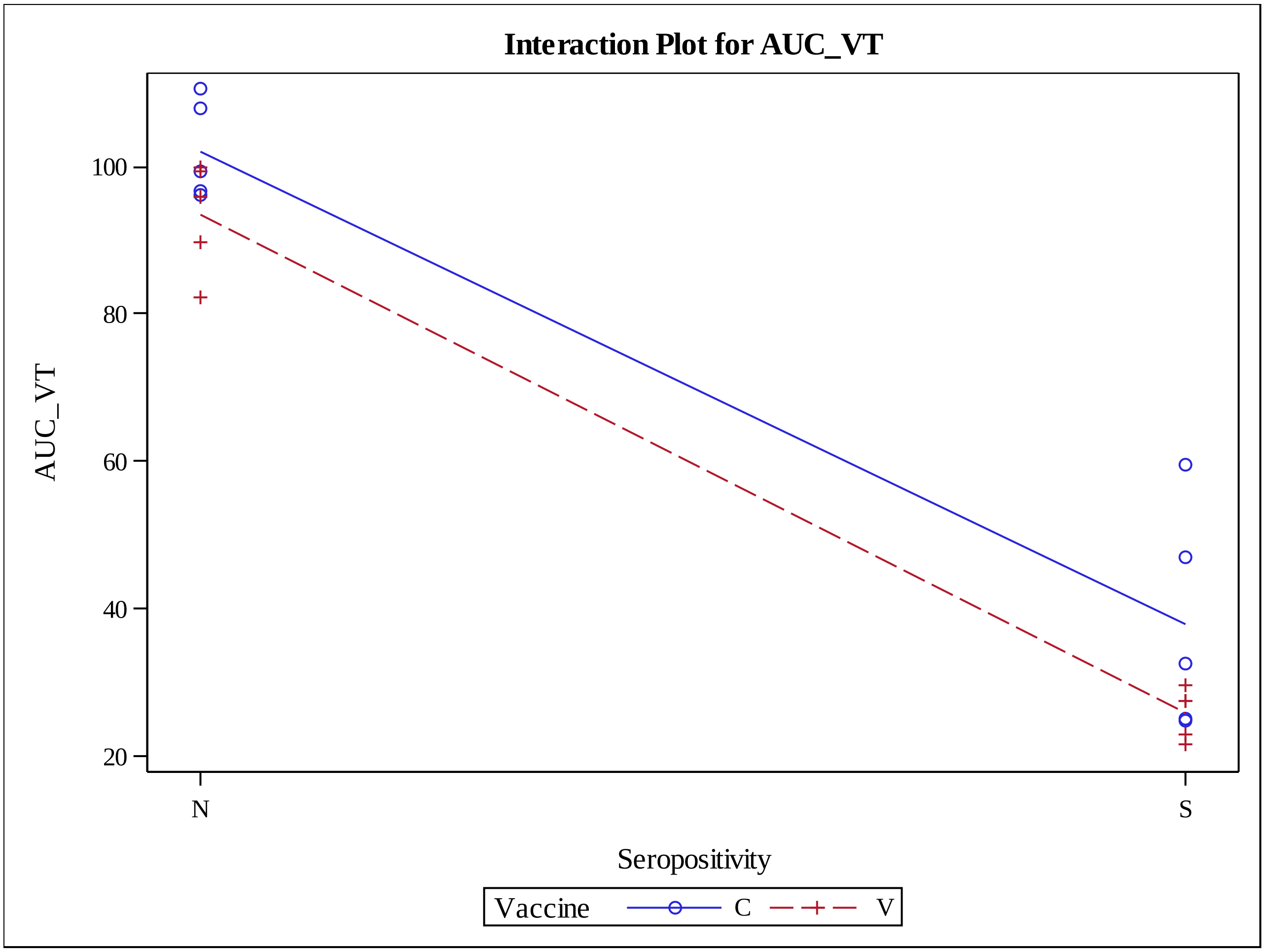
R-Square	**Coeff Var**	**Root MSE**	**AUC_VT Mean**
0.942841	14.17659	9.187830	64.80987

Source	**DF**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
Seropositivity	1	21732.58209	21732.58209	257.45	<.0001
Vaccine	1	533.73977	533.73977	6.32	0.0230
Seropositivi*Vaccine	1	12.74605	12.74605	0.15	0.7027

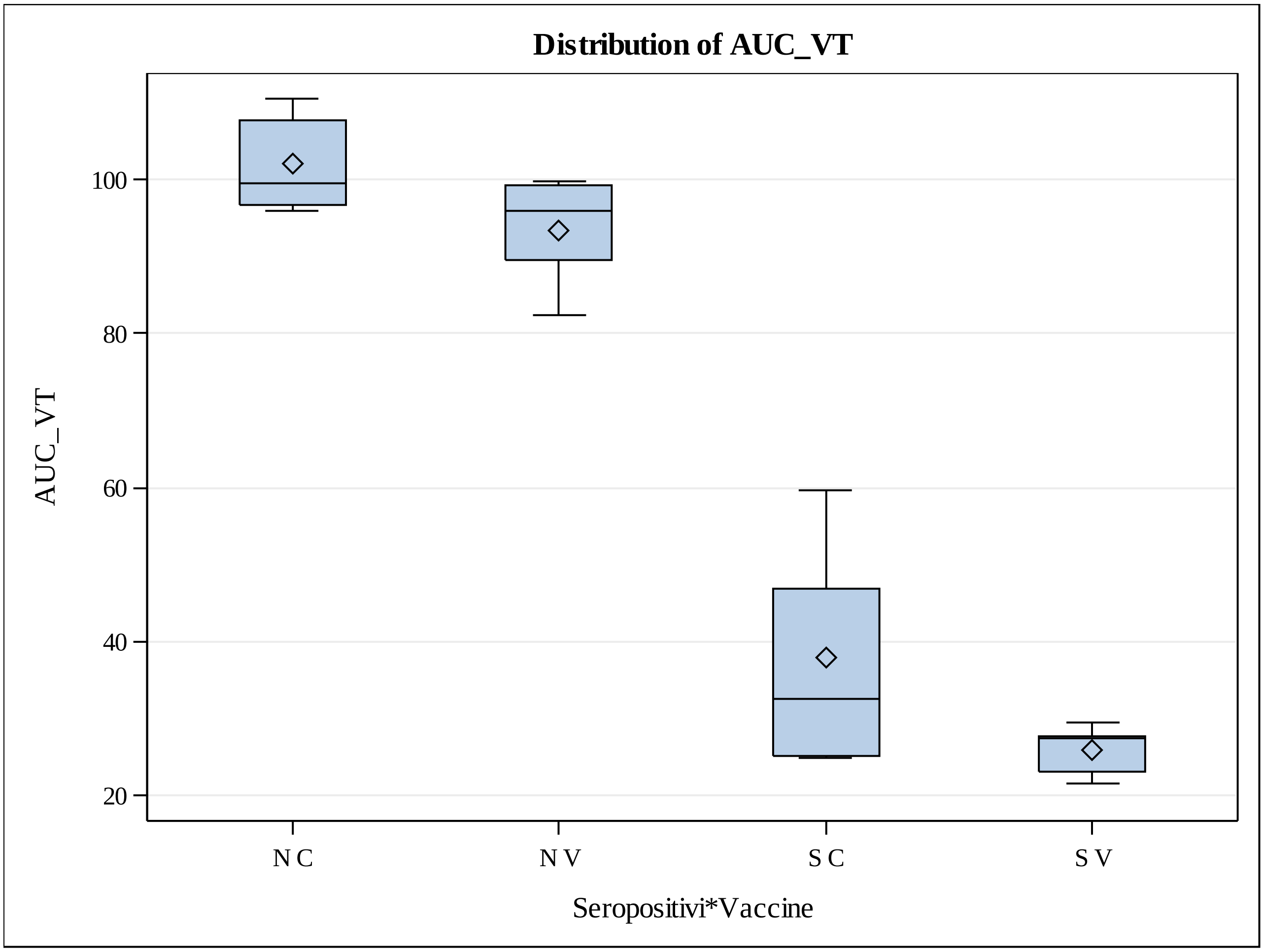
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Seropositivity	1	21732.58209	21732.58209	257.45	<.0001
Vaccine	1	533.73977	533.73977	6.32	0.0230
Seropositivi*Vaccine	1	12.74605	12.74605	0.15	0.7027

The GLM Procedure

Dependent Variable: AUC_VT



The GLM Procedure



Level of Seropositivity	Level of Vaccine	N	AUC_VT	
			Mean	**Std Dev**
N	**C**	5	102.141559	6.6424966
N	**V**	5	93.406293	7.3982536
S	**C**	5	37.810067	15.0820632
S	**V**	5	25.881553	3.3673947

The GLM Procedure

Class Level Information		
Class	**Levels**	**Values**
Seropositivity	2	N S
Vaccine	2	C V

Number of Observations Read	20
Number of Observations Used	20

The GLM Procedure

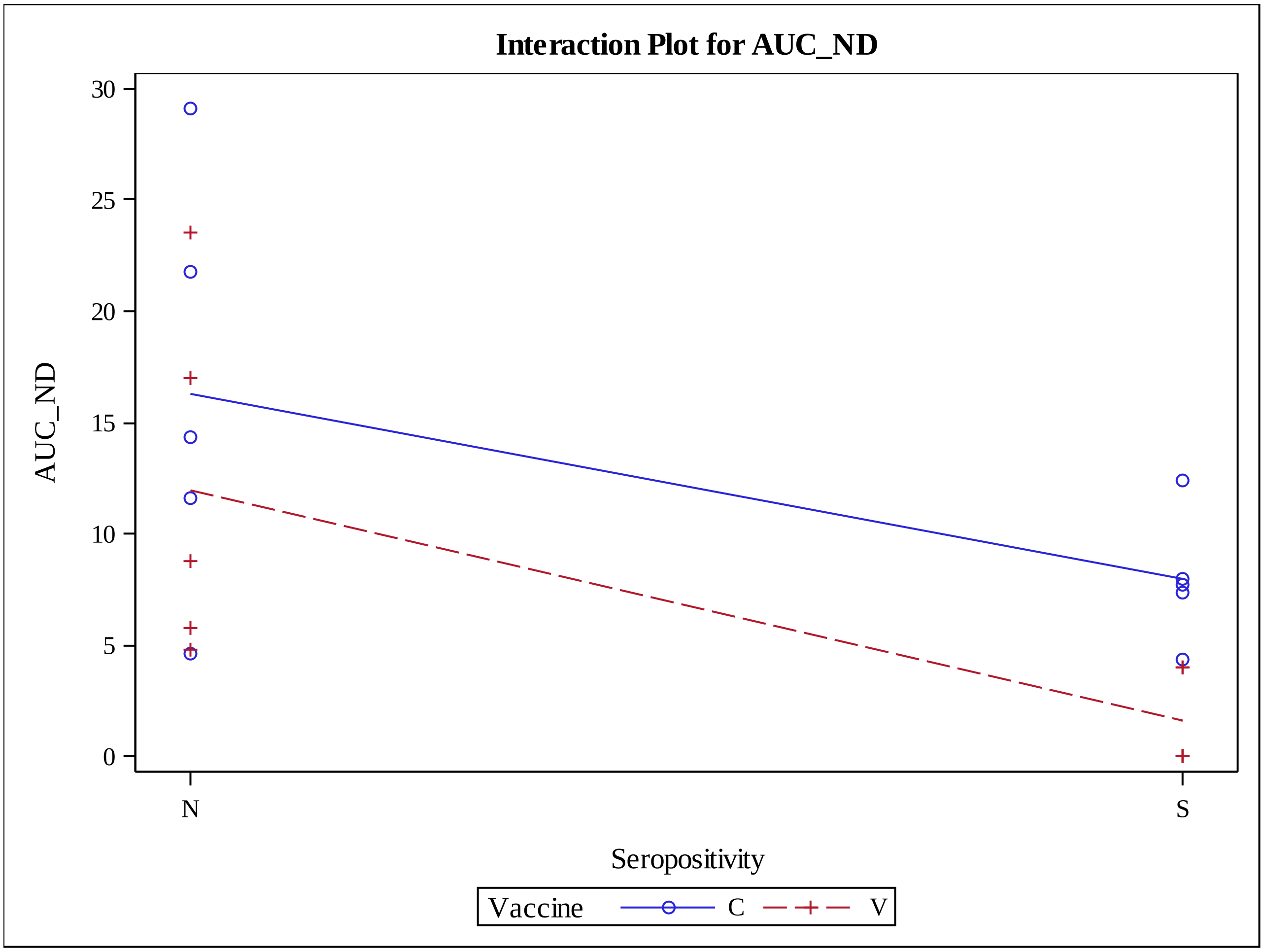
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	584.852344	194.950781	4.67	0.0157
Error	16	667.406250	41.712891		
Corrected Total	19	1252.258594			

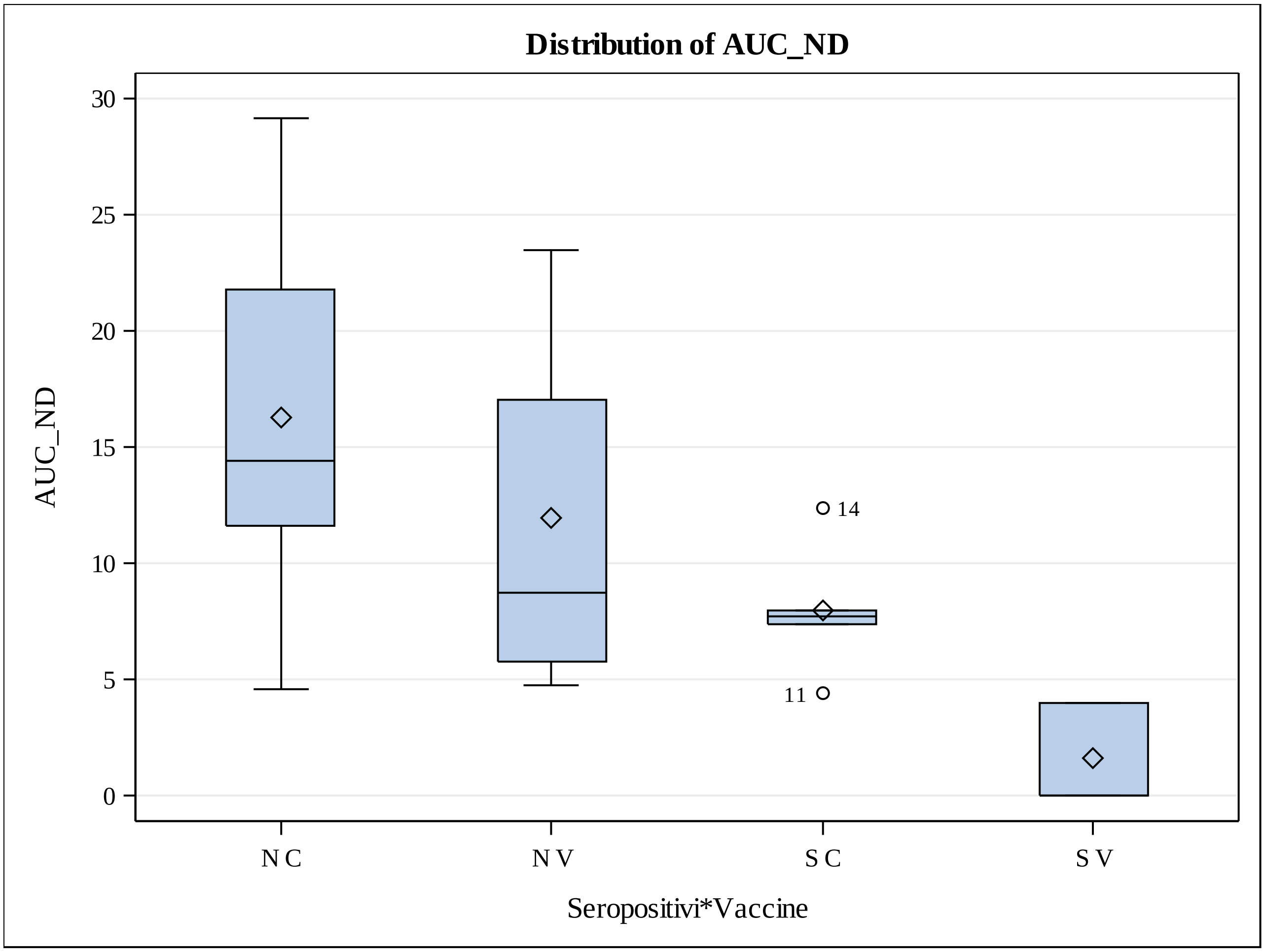
R-Square	Coeff Var	Root MSE	AUC_ND Mean
0.467038	68.29929	6.458552	9.456250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Seropositivity	1	435.9445313	435.9445313	10.45	0.0052
Vaccine	1	143.7820313	143.7820313	3.45	0.0819
Seropositivi*Vaccine	1	5.1257813	5.1257813	0.12	0.7305

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Seropositivity	1	435.9445313	435.9445313	10.45	0.0052
Vaccine	1	143.7820313	143.7820313	3.45	0.0819
Seropositivi*Vaccine	1	5.1257813	5.1257813	0.12	0.7305

The GLM Procedure





Level of Seropositivity	Level of Vaccine	N	AUC_ND	
			Mean	Std Dev
N	**C**	5	16.3000000	9.43464361
N	**V**	5	11.9500000	8.05333782
S	**C**	5	7.9750000	2.86056157
S	**V**	5	1.6000000	2.19089023

Table 1: Cohen's f^2 the effect size of virus titre post challenge by grouping factors and day post challenge.

Cohen's f^2	Grouping factor	Day post challenge
0.128	Seropositivity	1
1.714	Seropositivity	2
4.055	Seropositivity	3
11.069	Seropositivity	4
4.787	Seropositivity	5
9.636	Seropositivity	6
8.912	Seropositivity	7
1.805	Seropositivity	8
1.623	Seropositivity	9
2.916	Seropositivity	10
0.826	Seropositivity	11
2.957	Seropositivity	12
0.621	Seropositivity	13
0.002	Seropositivity	14
0.321	Vaccine	1
0.186	Vaccine	2
0.013	Vaccine	3
0.018	Vaccine	4
0.364	Vaccine	5
0.324	Vaccine	6
0.446	Vaccine	7
0.165	Vaccine	8
0.315	Vaccine	9
0.060	Vaccine	10
0.341	Vaccine	11
0.827	Vaccine	12
0.016	Vaccine	13
0.001	Vaccine	14

Table 2: Cohen's f^2 the effect size of nasal discharge score by grouping factors and day post challenge

Cohen's f2	Grouping factor	Day post challenge
	Seropositivity	1
	Seropositivity	2
0.109	Seropositivity	3
0.038	Seropositivity	4
0.265	Seropositivity	5
0.097	Seropositivity	6

0.157	Seropositivity	7
2.156	Seropositivity	8
0.787	Seropositivity	9
0.782	Seropositivity	10
0.780	Seropositivity	11
0.432	Seropositivity	12
0.128	Seropositivity	13
0.419	Seropositivity	14
	Vaccine	1
	Vaccine	2
0.002	Vaccine	3
0.002	Vaccine	4
0.135	Vaccine	5
1.326	Vaccine	6
2.395	Vaccine	7
0.025	Vaccine	8
0.205	Vaccine	9
0.070	Vaccine	10
0.047	Vaccine	11
0.169	Vaccine	12
0.009	Vaccine	13
0.000	Vaccine	14

Cohen’s f^2 is used to measure the practical significance of our test, we report the local effect size Cohen’s f^2 for repeated-measures[1].

Reference:

1- Selya, A. S., Rose, J. S., Dierker, L. C., Hedeker, D., & Mermelstein, R. J. (2012). A Practical Guide to Calculating Cohen's f(2), a Measure of Local Effect Size, from PROC MIXED. *Frontiers in psychology*, *3*, 111. doi:10.3389/fpsyg.2012.00111