Appendix

Supplementary Methods

Plaque Reduction Neutralization Test

Serum samples were analyzed by Battelle Biomedical Research Center (West Jefferson, OH). Briefly, heat-inactivated sera were serially diluted and incubated with WEEV (wild-type strain Fleming), VEEV (wild-type strain Trinidad Donkey), or EEEV (wild-type strain V105-00210) alphavirus stocks for approximately one hour. The virus-serum mixture was added to confluent monolayers of VERO E6 cells in triplicate and further incubated for approximately one hour after which overlay media was added. After approximately 48 hours of incubation, the overlay media was removed, and the cells were stained with crystal violet and fixed with a formalin solution. Plaques were counted manually and the reciprocal of serum dilutions that neutralize 80% of input virus ($PRNT_{80}$ titers) were measured. The average PRNT titers were calculated using STATA® software program for each sample tested.

Table 1: Maximum local and systemic solicited reactogenicity

	6 mcg		6 mcg+alum		30 mcg		30 mcg+alum		60 mcg		60 mcg+alum		Overall (*n* = 30)
	Dose 1 (*n* = 5)	Dose 2 (*n* = 4)	Dose 1 (*n* = 5)	Dose 2 (*n* = 5)	Dose 1 (*n* = 5)	Dose 2 (*n* = 5)	Dose 1 (*n* = 5)	Dose 2 (*n* = 5)	Dose 1 (*n* = 5)	Dose 2 (*n* = 5)	Dose 1 (*n* = 5)	Dose 2 (*n* = 5)	
Local *Symptoms*							***n* (%)**						
Pain/ Tenderness													
None	4 (80)	3 (75)	4 (80)	2 (40)	2 (40)	0 (0)	2 (40)	1 (20)	1 (20)	2 (40)	1 (20)	1 (20)	8 (27)
Mild	1 (20)	1 (25)	1 (20)	3 (60)	3 (60)	5 (100)	3 (60)	4 (80)	4 (80)	3 (60)	4 (80)	4 (80)	22 (73)
Swelling													
None	5 (100)	4 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	30 (100)
Redness													
None	5 (100)	4 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	30 (100)
Any													
None	4 (80)	3 (70)	4 (80)	2 (40)	2 (40)	0 (0)	2 (40)	1 (20)	1 (20)	2 (40)	1 (20)	1 (20)	8 (27)
Mild	1 (20)	1 (25)	1 (20)	3 (60)	3 (60)	5 (100)	3 (60)	4 (80)	4 (80)	3 (60)	4 (80)	4 (80)	22 (73)
Systemic *symptoms*							***n* (%)**						
Malaise													
None	4 (80)	4 (80)	4 (80)	4 (80)	3 (60)	3 (60)	3 (60)	2 (40)	3 (60)	4 (80)	2 (40)	2 (40)	15 (50)
Mild	1 (20)	0 (0)	1 (20)	1 (20)	2 (40)	2 (40)	2 (40)	3 (60)	2 (40)	1 (20)	3 (60)	3 (60)	15 (50)
Myalgia													
None	5 (100)	4 (100)	4 (80)	5 (100)	4 (80)	4 (80)	5 (100)	3 (60)	4 (80)	4 (80)	3 (60)	3 (60)	21 (70)
Mild	0 (0)	0 (0)	1 (20)	0 (0)	1 (20)	1 (20)	0 (0)	2 (40)	1 (20)	1 (20)	2 (40)	2 (40)	9 (30)
Headache													
None	4 (80)	3 (75)	4 (80)	4 (80)	3 (60)	4 (80)	3 (60)	4 (80)	4 (80)	5(100)	3 (60)	4 (80)	21 (70)
Mild	1 (20)	1 (25)	1 (20)	1 (20)	2 (40)	1 (20)	1 (20)	1 (20)	1 (20)	0 (0)	2 (40)	0 (0)	7 (23)
Moderate	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	2 (7)
Chills													
None	5 (100)	4 (100)	4 (80)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	29 (97)
Mild	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)
Nausea													
None	4 (80)	4 (100)	3 (60)	5 (100)	5 (100)	5 (100)	5 (100)	4 (80)	5 (100)	4 (80)	4 (80)	4 (80)	24 (80)

Mild	1 (20)	0 (0)	2 (40)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	1 (20)	1 (20)	1 (20)	6 (20)
Temperature													
None	5 (100)	4 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	30 (100)
Joint pain													
None	5 (100)	4 (100)	5 (100)	5 (100)	4 (80)	5 (100)	4 (80)	5 (100)	5 (100)	5 (100)	5 (100)	4 (80)	27 (90)
Mild	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	3 (10)
Any													
None	3 (60)	3 (75)	3 (60)	4 (80)	3 (60)	3 (60)	3 (60)	1 (20)	2 (40)	3 (60)	1 (20)	2 (40)	11 (37)
Mild	2 (40)	1 (25)	2 (40)	1 (20)	2 (40)	2 (40)	1 (20)	4 (80)	3 (60)	2 (40)	4 (80)	2 (40)	17 (57)
Moderate	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	2 (7)

Table 2: Comparison of the percentage of participants experiencing solicited reactogenicity post either dose

		Solicited Reactogenicity by Dose Level						Solicited Reactogenicity by Adjuvant		
Symptom	**Statistic**	**6 mcg WEVEE Alone and WEVEE + Alum *(n = 10)***	**30 mcg WEVEE Alone and WEVEE + Alum *(n = 10)***	**60 mcg WEVEE Alone and WEVEE + Alum *(n = 10)***	**Difference between 30 mcg and 6 mcg Groups**	**Difference between 60 mcg and 6 mcg Groups**	**Difference between 60 mcg and 30 mcg Groups**	**All Dose Levels (6, 30, and 60 mcg) WEVEE Alone *(n = 15)***	**All Dose Levels (6, 30, and 60 mcg) WEVEE + Alum *(n = 15)***	**Difference between All Dose Levels + Alum and All Dose Levels Alone**
Any symptom	n (%)	6 (60)	10 (100)	9 (90)	4 (40)	3 (30)	-1 (-10)	11 (73)	14 (93)	3 (20)
	95% CI	26, 88	69, 100	55, 100	10, 70	-6, 66	-29, 9	45, 92	68, 100	-6, 46
Pain/ Tenderness	n (%)	4 (40)	9 (90)	9 (90)	5 (50)	5 (50)	0 (0)	10 (67)	12 (80)	2 (13)
	95% CI	12, 74	55, 100	55, 100	14, 86	14, 86	-26, 26	38, 88	52, 96	-18, 45
Malaise	n (%)	3 (30)	6 (60)	6 (60)	3 (30)	3 (30)	0 (0)	5 (33)	10 (67)	5 (33)
	95% CI	7, 65	26, 88	26, 88	-12, 72	-12, 72	-43, 43	12, 62	38, 88	0, 67
Myalgia	n (%)	1 (10)	3 (30)	5 (50)	2 (20)	4 (40)	2 (20)	3 (20)	6 (40)	3 (20)
	95% CI	0, 45	7, 65	19, 81	-14, 54	4, 76	-22, 62	4, 48	16, 68	-12, 52
Headache	n (%)	2 (20)	4 (40)	3 (30)	2 (20)	1 (10)	-1 (-10)	4 (27)	5 (33)	1 (7)
	95% CI	3, 56	12, 74	7, 65	-19, 59	-28, 48	-52, 32	8, 55	12, 62	-26, 39
Chills	n (%)	1 (10)	0 (0)	0 (0)	-1 (-10)	-1 (-10)	0 (0)	0 (0)	1 (7)	1 (7)
	95% CI	0, 45	0, 31	0, 31	-29, 9	-29, 9	NC	0, 22	0, 32	-6, 19
Nausea	n (%)	3 (30)	1 (10)	2 (20)	-2 (-20)	-1 (-10)	1 (10)	2 (13)	4 (27)	2 (13)
	95% CI	7, 65	0, 45	3, 56	-54,14	-48, 28	-21, 41	2, 40	8, 55	-15, 42
Joint pain	n (%)	0 (0)	2 (20)	1 (10)	2 (20)	1 (10)	-1 (-10)	1 (7)	2 (13)	1 (7)
	95% CI	0, 31	3, 56	0, 45	-5, 45	-9, 29	-41, 21	0, 32	2, 40	-15, 28

NC= Not Calculable

Table 3: Geometric Mean Titers (GMT) of $PRNT_{80}$ titers by group and percent positive responders[1]

	Week 0 Baseline	Week 4 4 weeks post 1st vac	Week 10 2 weeks post 2nd vac	Week 12 4 weeks post 2nd vac	Week 24 16 weeks post 2nd vac	Week 36 28 weeks post 2nd vac
WEVEE VLP dose			*GMT (95% CI) [% responders]*			
6 mcg (*n* = 4)						
EEEV	5 [0%]	6·2 (3·1, 12·5) [25%]	6·9 (2·5, 19·1) [25%]	7·8 (1·9, 31·4) [25%]	5 [0%]	6·5 (2·9, 14·7) [25%]
VEEV	5 [0%]	6·5 (2·9, 14·7) [25%]	9·3 (2·9, 29·6) [50%]	10·9 (4·3, 27·8) [75%]	5 [0%]	6·9 (2·5, 19·1) [25%]
WEEV	5 [0%]	6·8 (2·6, 18·0) [25%]	9·7 (1·2, 79·0) [25%]	17·8 (1·7, 191·0) [50%]	5 [0%]	8·4 (1·6, 44·0) [25%]
6 mcg + alum (*n* = 5)						
EEEV	5 [0%]	5 [0%]	33·4 (13·5, 82·5) [100%]	33·0 (9·7, 112·6) [100%]	8·4 (2·0, 35·9) [20%]	8·1 (2·1, 31·4) [20%]
VEEV	5 [0%]	19·0 (6·9, 52·5) [80%]	42·5 (5·9, 305·3) [80%]	23·3 (2·9, 185·0) [60%]	14·7 (2·8, 75·9) [60%]	8·4 (2·9, 24·6) [20%]
WEEV	5 [0%]	5 [0%]	107·1 (38·2, 300·4) [100%]	138·5 (42·5, 451·3) [100%]	21·6 (6·2, 75·3) [80%]	8·9 (4·2, 19·1) [40%]
30 mcg (*n* = 5)						
EEEV	5 [0%]	5 [0%]	62·4 (21·4, 181·9) [100%]	14·4 (5·1, 41·2) [80%]	18·4 (4·1, 82·2) [60%]	20·1 (6·2, 65·0) [80%]
VEEV	5 [0%]	6·3 (3·3, 12·0) [20%]	58·6 (29·0, 118·5) [100%]	49·9 (30·7, 80·9) [100%]	26·2 (15·9, 43·1) [100%]	12·3 (3·9, 39·0) [60%]
WEEV	5 [0%]	8·0 (3·6, 17·6) [40%]	39·1 (6·8, 226·3) [80%]	50·3 (16·8, 150·2) [100%]	15·7 (3·3, 74·0) [60%]	6·9 (2·8, 16·9) [20%]
30 mcg + alum (*n* = 5)						
EEEV	5 [0%]	5 [0%]	133·4 (73·6, 241·5) [100%]	60·8 (29·9, 124·0) [100%]	53·4 (22·4, 127·3) [100%]	32·3 (16·1, 65·0) [100%]
VEEV	5 [0%]	27·4 (17·8, 42·2) [100%]	166·4 (59·2, 468·8) [100%]	111·5 (49·8, 249·8) [100%]	44·3 (15·6, 126·1) [100%]	16·4 (3·0, 88·8) [60%]
WEEV	5 [0%]	26·1 (6·4, 107·5) [80%]	170·0 (103·8, 278·5) [100%]	187·9 (90·0, 392·2) [100%]	31·0 (8·2, 116·7) [80%]	13·5 (6·3, 28·5) [80%]

60 mcg (*n* = 5)						
EEEV	5 [0%]	5 [0%]	92·9 (37·4, 230·9) [100%]	39·6 (19·6, 80·1) [100%]	31·8 (5·8, 173·5) [80%]	18·3 (3·7, 91·3) [60%]
VEEV	5 [0%]	11·4 (3·7, 35·5) [60%]	146·5 (87·4, 245·6) [100%]	110·5 (49·8, 245·5) [100%]	49·8 (28·9, 85·7) [100%]	22·6 (12·3, 41·5) [100%]
WEEV	5 [0%]	42·4 (31·7, 56·9) [100%]	27·8 (5·5, 141·2) [80%]	88·2 (37·7, 206·4) [100%]	17·3 (4·1, 72·9) [60%]	15·1 (4·0, 56·2) [60%]
60 mcg + alum (*n* = 5)						
EEEV	5 [0%]	5 [0%]	82·5 (35·9, 189·2) [100%]	24·4 (7·3, 81·3) [80%]	50·6 (22·2, 115·0) [100%]	22·2 (6·0, 82·9) [80%]
VEEV	5 [0%]	40·8 (16·2, 102·6) [100%]	85·4 (37·2, 195·8) [100%]	68·7 (34·8, 135·7) [100%]	42·3 (20·0, 89·5) [100%]	11·9 (4·0, 35·9) [60%]
WEEV	5 [0%]	18·6 (4·1, 84·4) [60%]	24·9 (5·3, 117·4) [80%]	93·2 (41·4, 209·6) [100%]	51·7 (19·4, 137·9) [100%]	24·0 (6·5, 88·4) [80%]

[1]Positive Responders are defined as responses above the assay limit of detection ($PRNT_{80}$ > 10). All pre-vaccination samples were tested negative and below the assay limit of detection; the background titers are shown as 5 (50% of the assay limit of detection) for statistical evaluation purposes.
Study vaccinations occurred at week 0 (baseline) and week 8.

Table 4: Positive responders[1] by dose group and vaccine antigen

	Week 0 Baseline	**Week 4 4 weeks post 1st vac**	**Week 10 2 weeks post 2nd vac**	**Week 12 4 weeks post 2nd vac**	**Week 24 16 weeks post 2nd vac**	**Week 36 28 weeks post 2nd vac**
	N (%)					
WEVEE VLP dose group	***Positive responders to all three vaccine antigens***					
6 mcg *(n=4)*	0 (0)	0 (0)	1 (25)	1 (25)	0 (0)	1 (25)
6 mcg + alum *(n=5)*	0 (0)	0 (0)	4 (80)	3 (60)	1 (20)	1 (20)
30 mcg *(n=5)*	0 (0)	0 (0)	4 (80)	4 (80)	2 (40)	1 (20)
30 mcg + alum *(n=5)*	0 (0)	0 (0)	5 (100)	5 (100)	4 (80)	2 (40)
60 mcg *(n=5)*	0 (0)	0 (0)	4 (80)	5 (100)	3 (60)	2 (40)
60 mcg + alum *(n=5)*	0 (0)	0 (0)	4 (80)	4 (80)	5 (100)	2 (40)
All dose groups combined						
EEEV *(n=29)*	0 (0)	1 (3.4)	26 (89.7)	24 (82.8)	18 (62.1)	18 (62.1)
VEEV *(n=29)*	0 (0)	19 (65.5)	26 (89.7)	26 (89.7)	23 (79.3)	16 (55.2)
WEEV *(n=29)*	0 (0)	15 (51.7)	23 (79.3)	27 (93.1)	19 (65.5)	15 (51.7)
All antigens *(n=29)*	0 (0)	0 (0)	22 (75.9)	22 (75.9)	15 (51.7)	9 (31)

[1]Positive Responders are defined as responses above the assay limit of detection ($PRNT_{80}$ > 10) to all three vaccine components. All pre-vaccination samples were tested negative and below the assay limit of detection.
Study vaccinations occurred at week 0 (baseline) and week 8.