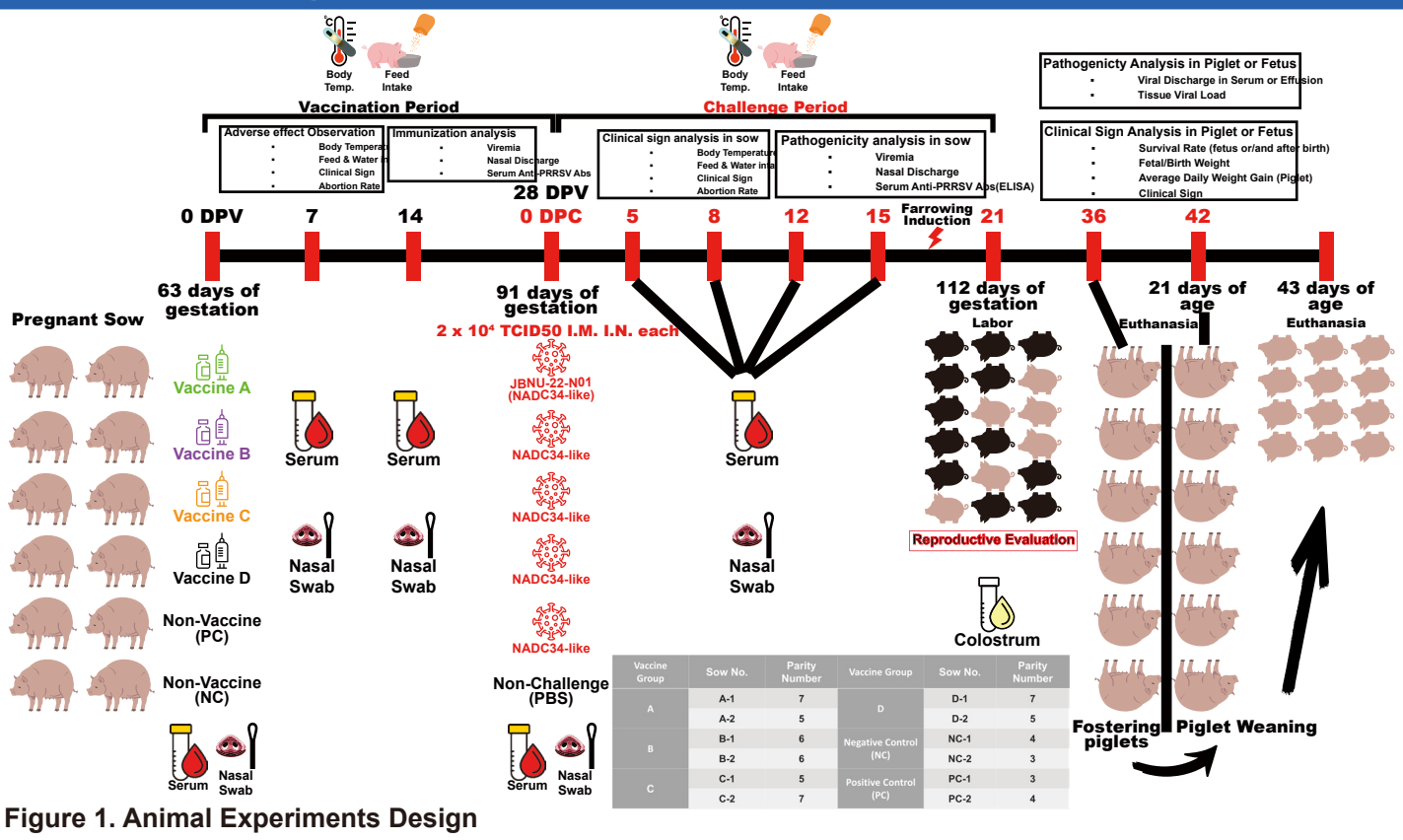
Supplementary



Vaccine Group	Sow No.	Parity Number	Vaccine Group	Sow No.	Parity Number
A	A-1	7	D	D-1	7
	A-2	5		D-2	5
B	B-1	6	Negative Control (NC)	NC-1	4
	B-2	6		NC-2	3
C	C-1	5	Positive Control (PC)	PC-1	3
	C-2	7		PC-2	4

Figure 1. Animal Experiments Design

Twelve pregnant sows were divided into 6 groups: Negative control (NC), Positive control (PC), and Vaccines A-D (**Table 1a**). At 63 days of gestation, sows in the vaccine groups were administered their respective vaccines according to manufacturers' instructions. Serum and nasal swab samples were collected on the day of vaccination and at 7, 14, and 28 days post-vaccination (DPV). Body temperature and feed intake were monitored TID.

At 91 days of gestation, the PC and vaccinated groups were challenged with a Korean NADC34-like PRRSV strain (JBNU-22-N01, **Table 1b**). Serum and nasal swab samples were collected at 5, 8, 12, and 15 days post-challenge (DPC).

At 111 days of gestation (20 DPC), all sows received PGF2α for labor induction and parturition occurred within 1-2 days. Abortion rates were evaluated post-farrowing, and surviving piglets were monitored through 43 days of age to assess survival rates and average daily weight gain during the suckling period (4-21 days of age). At approximately 15 days post-farrowing, half of the sows from each group were euthanized, and their piglets were cross-fostered to remaining sows within the same treatment group. At 21 days of birth (weaning), remaining sows were euthanized. All surviving piglets from the PC and vaccinated groups were housed separately by treatment group until 43 days of age, while 3 piglets were randomly selected from the NC group for continued monitoring. All monitored piglets were euthanized and necropsied at 43 days of age for final evaluation.

Table 1. Information fo Vaccines and Virus Used in This Study

a)

Vaccine	Lineage (ORF5)	Approved Year (In South Korea)	Dose	ORF5 sequence Homology Against Challenged Virus	Notes
A	L1A*	Candidate	10^5 $TCID_{50}$/dose	N/A	Chimeric, codon pair deoptimized(CPD) live vaccine with *ORF5 sequence replaced from Korean NADC34-like virus
B	L1A**	Candidate	2 x $10^{5.5}$ $TCID_{50}$/dose	100%	Infectious clone-based live vaccine (Ribavirin-Resistant PRRS(RVRp22)) **ORF5-6 sequence replaced from JBNU-22-N01
C	L1J	2023 (Candidate)	10^5 $TCID_{50}$/dose 10^4 $TCID_{50}$/dose	86.1%	Live attenuated vaccine High dose / Low dose
D	L5	1995	$10^{4.8}$ $TCID_{50}$/dose	83.5%	Live attenuated vaccine

b)

Strain	Dose	Notes
JBNU-22-N01	2ml x 10^4 $TCID_{50}$/ml I.M. and I.N. each	Genebank Accession No. OP970983

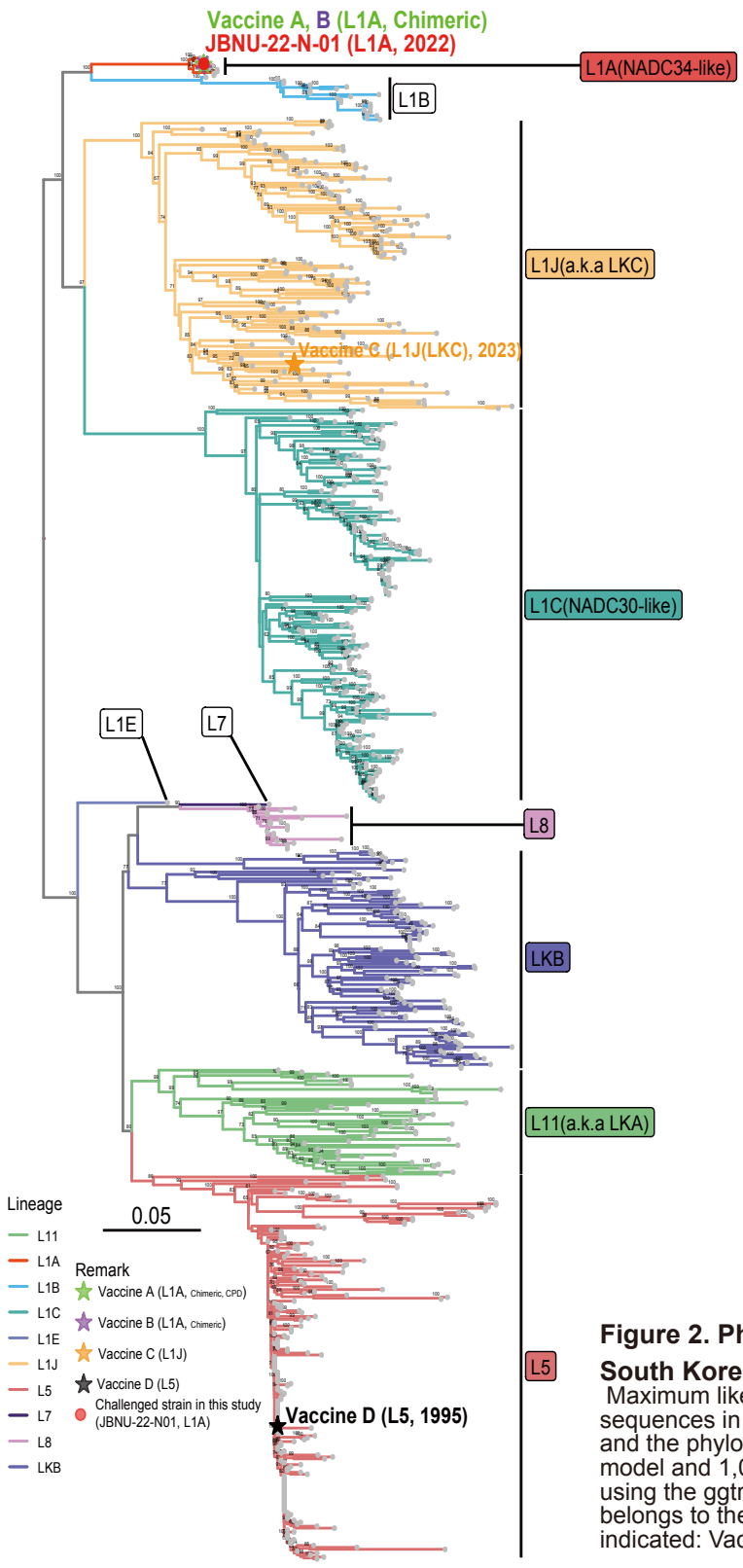


Figure 2. Phylogenetic analysis of PRRSV-2 ORF5 Sequences in South Korea (-2022)

Maximum likelihood phylogenetic tree was constructed from 781 PRRSV-2 ORF5 sequences in South Korea. Sequence alignment was performed using MAFFT, and the phylogenetic tree was constructed using IQ-TREE2 with the GTR+F model and 1,000 ultra-fast bootstrap replicates. Tree visualization was performed using the ggtree2 package in R. The challenge virus JBNU-22-N01 (red circle) belongs to the L1A (NADC34-like). Vaccine strains used in this study are indicated: Vaccine A and B (L1A), Vaccine C (L1J), and Vaccine D (L5).