Table S1. Specimen-level metadata and isolation outcomes (15 clinical specimens, Central Federal District, February 2025).

Specimen no.	Farm	Animal group	Specimen type	Collection date	Original Ct	Freeze–thaw history	Inoculated onto	Isolation outcome (PAM, 3 passages)
1	1	Sow (tested)	Blood	Feb 2025	17.77	1×, −20 °C	PAM	No isolate
2	1	Sow (tested)	Blood	Feb 2025	17.11	1×, −20 °C	PAM, PL, MARC-145	PRRSV/2025/1/2
3	1	Piglet 0–1 m	Blood	Feb 2025	17.70	1×, −20 °C	PAM, PL, MARC-145	PRRSV/2025/1/3
4	13	Piglet 0–1 m	Organs suspension	Feb 2025	21.83	1×, −20 °C	PAM	No isolate
5	6	Piglet 0–1 m	Blood	Feb 2025	17.58	1×, −20 °C	PAM, PL, MARC-145	PRRSV/2025/6/5
6	6	Piglet 0–1 m	Blood	Feb 2025	17.94	1×, −20 °C	PAM	No isolate
7	6	Piglet 0–1 m	Organs suspension	Feb 2025	22.11	1×, −20 °C	PAM	No isolate
8	6	Piglet 0–1 m	Blood	Feb 2025	19.98	1×, −20 °C	PAM	No isolate
9	6	Piglet 0–1 m	Organs suspension	Feb 2025	24.66	1×, −20 °C	PAM	No isolate
10	13	Sow (tested)	Blood	Feb 2025	20.58	1×, −20 °C	PAM, PL, MARC-145	PRRSV/2025/13/10
11	10	Piglet 0–1 m	Blood	Feb 2025	17.50	1×, −20 °C	PAM, PL, MARC-145	PRRSV/2025/10/11
12	9	Sow (main herd)	Blood	Feb 2025	34.67	1×, −20 °C	PAM	No isolate
13	7	Sow (tested)	Organs suspension	Feb 2025	Negative	1×, −20 °C	Not inoculated	—
14	7	Sow (main herd)	Organs suspension	Feb 2025	16.53	1×, −20 °C	PAM	No isolate
15	7	Piglet 0–1 m	Organs suspension	Feb 2025	17.18	1×, −20 °C	PAM	No isolate

Table S2. RT-qPCR Ct values and infectious titers of original specimens and PAM passages (4 wells/dilution, 3 independent experiments; "—" = not detected / no virus recovered).

Specimen no.	Original material Ct	PAM passage 1 Ct	PAM passage 3 Ct	Passage-3 titer, lg TCID50/cm3 (range of 3 experiments)	Isolate
1	17.77	26.64	—	—	No isolate
2	17.11	7.50	6.55	5.00–5.25	PRRSV/2025/1/2
3	17.70	10.55	6.49	5.00–5.25	PRRSV/2025/1/3
4	21.83	30.51	—	—	No isolate
5	17.58	10.17	6.50	4.75–5.00	PRRSV/2025/6/5
6	17.94	27.14	—	—	No isolate
7	22.11	—	—	—	No isolate
8	19.98	29.15	—	—	No isolate
9	24.66	—	—	—	No isolate
10	20.58	14.58	6.00	4.75–5.00	PRRSV/2025/13/10
11	17.50	13.17	5.90	4.75–5.00	PRRSV/2025/10/11
12	34.67	—	—	—	No isolate
14	16.53	31.73	—	—	No isolate
15	17.18	29.16	—	—	No isolate

Table S3. RT-qPCR Ct values of PL cultures inoculated with original filtered specimens (dilutions 10^{-1}–10^{-2}) and with PAM passage-1 material.

Inoculum (specimen / type)	PL passage 1 Ct	PL passage 3 Ct
2 (original filtrate)	21.43	10.13
3 (original filtrate)	23.09	12.67
5 (original filtrate)	20.71	11.36
10 (original filtrate)	25.34	15.12
11 (original filtrate)	22.17	13.53
2 (PAM passage 1)	12.53	8.55
3 (PAM passage 1)	17.28	12.44

5 (PAM passage 1)	16.33	9.96
10 (PAM passage 1)	18.11	9.62
11 (PAM passage 1)	17.62	9.12

Table S4. Individual daily rectal temperatures (°C) of the six experimentally infected piglets. Fever threshold ≥ 40.0 °C. Necropsy days: piglets 1–2 (day 7), 3–4 (day 14), 5–6 (day 21).

Day post-inoculation	Piglet 1	Piglet 2	Piglet 3	Piglet 4	Piglet 5	Piglet 6
0	39.3	39.4	39.5	39	39.4	39.6
1	40.3	40.4	40	40.2	40.2	40.6
2	39.7	40	39.1	39.4	39.2	40.2
3	39.5	39.6	39.7	39.6	39.4	41.3
4	40.6	40.9	40.5	39.8	40.5	40.3
5	40.1	41.2	41.3	40.3	40.6	40.8
6	39.8	39.5	39.4	40.2	39.7	40.5
7	39.7 (necropsy)	40.2 (necropsy)	40.3	39.8	40.1	40.3
8	—	—	39.6	39.5	40.5	41.2
9	—	—	40.1	40	40	40.2
10	—	—	39.6	39.3	39.5	40.5
11	—	—	39.4	39.8	39.2	41.3
12	—	—	39.8	39.3	39.6	40.1
13	—	—	39.8	39.8	39.2	40.2
14	—	—	39.4 (necropsy)	39.2 (necropsy)	39.5	39.8
15	—	—	—	—	39.2	39.7
16	—	—	—	—	39.4	39.5
17	—	—	—	—	39	39.2
18	—	—	—	—	38.9	39

19	—	—	—	—	39.4	39.5
20	—	—	—	—	38.9	39.9
21	—	—	—	—	39.5 (necropsy)	39.8 (necropsy)

Table S5. Individual clinical signs (days post-inoculation). Fever days derived from Table S4 (≥40.0 °C).

Clinical sign	Piglet 1	Piglet 2	Piglet 3	Piglet 4	Piglet 5	Piglet 6
Fever (≥40.0 °C), days with fever	1, 4, 5	1, 2, 4, 5, 7	1, 4, 5, 7, 9	1, 5, 6, 9	1, 4, 5, 7, 8, 9	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
Reduced appetite / activity	4–6 (3 d)	4–7 (4 d)	5–7 (3 d)	5–6 (2 d)	6–9 (4 d)	4–14 (11 d)
CNS signs (muscular tremor)	—	—	1–3, 8–9	—	—	1–4, 8–11
Respiratory signs	—	—	—	—	—	—
Skin lesions	—	—	—	—	—	—

Table S6. Individual viremia data by sampling day. "—" = not detected or animal already necropsied. Piglets 1–2 necropsied day 7; 3–4 day 14; 5–6 day 21.

Day post-inoculation	Method	Piglet 1	Piglet 2	Piglet 3	Piglet 4	Piglet 5	Piglet 6
3	RT-qPCR Ct (blood)	29.41	27.38	28.19	28.26	30.45	27.46
3	Titer in PL, lg TCID50/cm3	2.0	2.25	2.0	2.25	—	2.25
3	Titer in PAM, lg TCID50/cm3	2.25	2.25	2.25	2.25	—	2.5
7	RT-qPCR Ct (blood)	25.40	23.08	24.95	26.30	25.06	24.84
7	Titer in PL, lg TCID50/cm3	3.25	3.25	3.5	3.0	3.25	3.5
7	Titer in PAM, lg TCID50/cm3	3.25	3.75	3.5	3.25	3.5	3.75
10	RT-qPCR Ct (blood)	—	—	26.65	30.43	26.61	25.49
10	Titer in PL, lg TCID50/cm3	—	—	2.75	—	2.5	3.0
10	Titer in PAM, lg	—	—	3.0	—	2.5	3.5

	TCID50/cm3						
14	RT-qPCR Ct (blood)	—	—	29.71	37.24 (weak positive: Ct 35–40 with sigmoidal curve and valid internal controls, confirmed on retest; see Section 2.4)	29.54	24.28
14	Titer in PL, lg TCID50/cm3	—	—	2.0	—	2.0	3.25
14	Titer in PAM, lg TCID50/cm3	—	—	2.0	—	2.25	3.5
21	RT-qPCR Ct (blood)	—	—	—	—	30.42	28.04
21	Titer in PL, lg TCID50/cm3	—	—	—	—	—	—
21	Titer in PAM, lg TCID50/cm3	—	—	—	—	—	—