

Supplementary Material

Porcine Epidemic Diarrhea Virus Shedding and Antibody Response in Swine Farms: a Longitudinal Study

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Supplementary Figures

Supplementary Figure 1. Dynamic range of the standard curve for the quantitative PEDV *S1* gene-based qRT-PCR assay. The C_q values of the 10-fold stock PEDV ssRNA transcript ($2 \times 10^8 - 2 \times 10^0$ copies/ μ L) solutions are dependent on the log values of the transcript amounts.

Supplementary Tables

Supplementary Table 1. Order of appearance of PED symptoms in the different units of the farm.

Farm	DPI	Unit	Symptoms					
			diarrhea	vomiting	anorexia	dehydration	cachexia	agalactia
1	0	fattening A	x	x	x			
	1-2	gestation	x					
	1-2	delivery (litters)	x			x	x	
	1-2	delivery (sows)	x					x
	3	weaning	x					
	3	growing	x					
	5	fattening B	x					
2	0	fattening	x		x			
	3	gestation	x					
	3	delivery (litters)	x		x			
	3	delivery (sows)	x		x			x
	3	weaning	x					
	4-5	growing	x					
3	0	gestation	x		x			
	1	delivery (litters)	x		x	x		
	1	delivery (sows)	x					x
	5	weaning	x					
	5	growing	x					
	5	fattening	x					
4	0	delivery (litters)	x				x	
	0	delivery (sows)	x					
	3	gestation	x					

DPI (Days Post Infection): days from the first appearance of the infection in the farm, x: presence of symptoms.

Supplementary Table 2. Maximum fecal score and percentage of diarrheic animals per farm.

Farm	Sampling time	Date	Gestation unit		Delivery room				Weaning unit		Growing unit		Fattening unit A		Fattening unit B	
			Fecal score	Diarrheic animals (%)	Sows		Piglets		Fecal score	Diarrheic animals (%)	Fecal score	Diarrheic animals (%)	Fecal score	Diarrheic animals (%)	Fecal score	Diarrheic animals (%)
					Fecal score	Diarrheic animals (%)	Fecal score	Diarrheic animals (%)								
1	preliminary	13 Jan	4	21-50	2	6-20	4	>50	2	0-5	4	21-50	4	>50	0	0
	1	16 Jan	3	21-50	2	5	4	>50*	3	21-50	4	21-50	4	>50	0	0
	2	2 Feb	0	0	0	0	4	6-20*	0	0	0	0	2	0-5	2	6-20
	3	12 Feb	0	0	0	0	4	21-50	0	0*	0	0	0	0	2	21-50
	4	12 Mar	0	0	0	0	0	0	2	0-5*	2	0-5	2	0-5	2	0-5
	5	17 Apr	0	0	0	0	0	0	0	0	0	0*	0	0	0	-
	6	4 June	0	0	0	0	0	0	0	0	0	0	0	0*	0	-*
2	preliminary	31 Jan	4	6-20	2	0-5	4	>50	4	6-20	4	6-20	4	6-20	/	/
	1	3 Feb	3	0-5	0	0	4	21-50*	3	0-5	3	0-5	4	6-20	/	/
	2	18 Feb	0	0	2	0-5	2	0-5*	0	0	0	0	0	0	/	/
	3	4 Mar	0	0	0	0	0	0	0	0*	0	0	0	0	/	/
	4	8 Apr	0	0	0	0	0	0	0	0*	0	0	0	0	/	/
	5	14 May	0	0	0	0	0	0	0	0	0	0*	0	0	/	/
3	preliminary	18 Feb	4	0-5	4	0-5	4	6-20	0	0	0	0	0	0	/	/
	1	20 Feb	4	6-20	4	21-50	4	>50*	2	0-5	4	0-5	4	0-5	/	/
	2	5 Mar	0	0	0	0	0	0*	0	0	0	0	0	0	/	/
	3	24 Mar	0	0	0	0	0	0	0	0	0	0	0	0	/	/
	4	29 Apr	0	0	0	0	0	0	0	0	0	0	0	0	/	/
4	preliminary	18 May	2	0-5	2	0-5	3	6-20	0	0	/	/	/	/	/	/
	1	6 June	0	0	4	21-50	4	21-50*	0	0	/	/	/	/	/	/
	2	17 June	0	0	2	0-5	0	0*	0	0	/	/	/	/	/	/
	3	23 July	0	0	0	0	0	0	0	0*	/	/	/	/	/	/
	4	5 Aug	0	0	0	0	0	0	0	0*	/	/	/	/	/	/

*indicates the location of animals involved in the study; “/” indicates that samples were not available

Supplementary Table 3. Symptoms observation, viral titers in fecal homogenates and cELISA results for animals involved in the longitudinal study.

Farm	Animal ID	1° SAMPLING			2° SAMPLING			3° SAMPLING			4° SAMPLING			5° SAMPLING			6° SAMPLING		
		Diarrhea	PEDv copies/mL	cELISA	Diarrhea	PEDv copies/mL	cELISA	Diarrhea	PEDv copies/mL	cELISA	Diarrhea	PEDv copies/mL	cELISA	Diarrhea	PEDv copies/mL	cELISA	Diarrhea	PEDv copies/mL	cELISA
1	1	+	<LOQ	+	NA	NA	+	+	-	+	-	-	+	-	-	+	-	-	+
1	2	-	-	-	NA	NA	+	-	-	+	-	-	+	-	-	+	-	-	+
1	3	+	-	-	NA	NA	+	-	-	+	-	-	+	-	-	+	-	-	+
1	4	-	2.00E+05	-	NA	NA	+	-	-	+	-	-	+	-	-	+	-	-	+
1	5	-	-	-	NA	NA	+	-	-	+	+	<LOD	+	-	-	+	-	-	+
1	6	+	1.00E+05	-	NA	NA	+	-	-	+	-	-	+	-	-	+	-	-	+
1	7	+	-	+	NA	NA	+	-	-	+	-	-	+	S	S	S	S	S	S
1	8	+	5.90E+05	-	NA	NA	+	-	-	+	-	-	+	-	-	+	S	S	S
1	9	+	1.20E+06	-	NA	NA	+	-	-	+	-	-	NA	-	-	+	S	S	S
1	10	+	9.50E+06	+	NA	NA	+	-	-	+	-	-	+	-	-	+	S	S	S
1	11	+	2.20E+05	-	NA	NA	+	D	D	D	D	D	D	D	D	D	D	D	D
1	12	+	4.60E+04	+	NA	NA	+	-	-	+	-	-	+	-	-	+	-	-	+
1	13	+	2.40E+07	+	NA	NA	+	-	-	+	NA	NA	NA	-	-	+	-	-	+
1	14	+	9.50E+04	-	NA	NA	+	-	-	+	-	4.90E+04	+	S	S	S	S	S	S
1	15	-	-	-	NA	NA	+	-	-	+	+	9.40E+05	+	S	S	S	S	S	S
1	16	+	-	-	NA	NA	-	-	-	+	+	7.90E+04	+	S	S	S	S	S	S
1	17	-	3.70E+07	-	NA	NA	+	-	-	+	-	-	+	-	<LOQ	+	S	S	S
1	18	+	2.10E+06	-	NA	NA	-	-	-	+	-	-	+	S	S	S	S	S	S
1	19	-	-	-	NA	NA	D	D	D	D	D	D	D	D	D	D	D	D	D
2	1	+	-	-	+	<LOQ	+	-	-	+	-	-	-	-	-	NA	/	/	/
2	2	+	3.30E+05	-	-	-	+	-	-	+	-	-	+	-	-	+	/	/	/
2	3	+	1.10E+06	-	-	-	+	-	-	+	-	-	+	-	-	+	/	/	/
2	4	+	1.10E+07	-	-	-	+	-	-	+	-	-	+	-	-	+	/	/	/
2	5	+	3.20E+04	-	-	-	+	-	-	+	-	-	+	-	-	+	/	/	/
2	6	+	3.30E+08	-	+	-	+	-	-	+	-	3.00E+04	+	-	-	+	/	/	/
2	7	+	1.20E+05	-	+	<LOQ	-	-	-	+	-	-	+	S	S	S	/	/	/
2	8	+	-	-	+	8.30E+05	-	-	-	+	D	D	D	D	D	D	/	/	/
2	9	+	7.00E+04	-	-	-	+	-	-	+	-	-	+	-	-	-	/	/	/
2	10	+	2.60E+04	-	-	-	+	-	-	+	D	D	D	D	D	D	/	/	/
2	11	+	7.00E+04	-	-	-	+	-	-	+	-	-	+	-	-	+	/	/	/
2	12	+	2.60E+04	-	-	-	+	-	-	+	-	-	+	-	-	+	/	/	/
2	13	+	7.00E+04	-	-	-	+	-	-	+	D	D	D	D	D	D	/	/	/
2	14	+	2.60E+04	-	+	-	+	-	-	+	-	-	+	-	-	+	/	/	/
2	15	+	-	-	-	-	+	-	-	+	-	-	+	-	-	+	/	/	/
2	16	+	7.30E+04	-	-	-	+	-	-	+	D	D	D	D	D	-	/	/	/

2	17	+	1.00E+04	-	-	-	+	-	-	+	-	-	+	-	-	+	/	/	/
2	18	+	1.40E+05	-	+	-	+	+	-	+	-	-	+	-	-	+	/	/	/
2	19	+	1.00E+08	-	-	-	+	-	-	+	-	-	+	-	-	+	/	/	/
2	20	+	4.50E+04	-	-	-	+	-	-	+	-	-	+	-	-	+	/	/	/
2	21	+	3.60E+05	-	-	-	+	-	-	+	-	-	+	S	S	S	/	/	/
2	22	+	< LOQ	-	-	-	+	-	-	+	-	-	+	-	-	-	/	/	/
2	23	+	1.30E+08	-	-	-	+	-	-	+	-	-	+	-	-	+	/	/	/
2	24	+	6.40E+06	-	+	< LOQ	+	+	1.10E+04	+	-	-	-	-	-	NA	/	/	/
3	1	+	7.80E+04	-	-	-	+	-	-	+	-	-	-	/	/	/	/	/	/
3	2	+	1.50E+06	-	-	-	+	D	D	D	D	D	D	/	/	/	/	/	/
3	3	+	3.50E+05	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
3	4	+	5.60E+06	-	-	-	+	-	-	+	S	S	S	/	/	/	/	/	/
3	5	+	6.80E+05	-	-	-	+	-	-	+	S	S	S	/	/	/	/	/	/
3	6	+	1.20E+04	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
3	7	+	9.10E+04	-	-	-	+	-	-	+	S	S	S	/	/	/	/	/	/
3	8	+	1.40E+04	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
3	9	+	< LOQ	-	-	-	+	-	-	+	S	S	S	/	/	/	/	/	/
3	10	+	5.10E+04	-	-	-	+	-	-	+	S	S	S	/	/	/	/	/	/
3	11	+	4.20E+05	-	-	-	+	-	-	+	S	S	S	/	/	/	/	/	/
3	12	+	5.40E+04	-	-	-	+	-	-	-	S	S	S	/	/	/	/	/	/
3	13	+	< LOQ	-	-	-	+	-	-	+	S	S	S	/	/	/	/	/	/
3	14	+	5.50E+06	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
3	15	+	2.70E+06	-	-	-	+	-	-	+	S	S	S	/	/	/	/	/	/
3	16	+	2.40E+06	-	-	-	-	-	-	+	S	S	S	/	/	/	/	/	/
3	17	+	1.20E+05	-	+	3.00E+05	+	-	-	+	-	-	+	/	/	/	/	/	/
3	18	+	1.50E+05	-	-	-	-	-	-	+	S	S	S	/	/	/	/	/	/
3	19	+	6.90E+05	-	-	-	-	-	-	+	-	-	+	/	/	/	/	/	/
3	20	+	2.50E+04	-	-	-	+	-	-	+	S	S	S	/	/	/	/	/	/
3	21	+	4.10E+05	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
3	22	+	6.40E+04	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
3	23	+	1.70E+05	-	-	-	-	-	-	+	-	-	+	/	/	/	/	/	/
3	24	+	1.10E+04	-	-	-	+	-	-	+	S	S	S	/	/	/	/	/	/
3	25	+	1.40E+06	-	+	1.10E+04	+	-	-	+	-	-	+	/	/	/	/	/	/
3	26	+	9.00E+06	-	+	6.70E+03	+	-	-	+	S	S	S	/	/	/	/	/	/
3	27	+	8.00E+05	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
3	28	+	1.50E+08	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
3	29	+	9.60E+05	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
3	30	+	4.00E+05	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
4	1	+	1.70E+07	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
4	2	+	5.90E+06	-	D	D	D	D	D	D	D	D	D	/	/	/	/	/	/
4	3	+	8.60E+06	-	D	D	D	D	D	D	D	D	D	/	/	/	/	/	/
4	4	+	1.10E+05	-	D	D	D	D	D	D	D	D	D	/	/	/	/	/	/

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4	5	+	6.20E+07	-	+	-	+	-	-	+	-	-	+	/	/	/	/	/	/
4	6	+	1.50E+04	-	+	1.80E+04	+	-	-	-	-	-	+	/	/	/	/	/	/
4	7	+	3.60E+07	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
4	8	+	2.40E+05	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
4	9	+	< LOQ	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
4	10	+	1.80E+05	-	D	D	D	D	D	D	D	D	D	/	/	/	/	/	/
4	11	+	9.80E+06	-	-	-	+	-	-	+	D	D	D	/	/	/	/	/	/
4	12	+	6.40E+05	-	D	D	D	D	D	D	D	D	D	/	/	/	/	/	/
4	13	+	1.40E+08	-	NA	NA	NA	NA	NA	NA	-	-	+	/	/	/	/	/	/
4	14	+	< LOQ	-	NA	NA	NA	NA	NA	NA	-	-	+	/	/	/	/	/	/
4	15	+	4.70E+07	-	NA	NA	NA	NA	NA	NA	-	-	+	/	/	/	/	/	/
4	16	+	1.20E+05	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
4	17	+	5.60E+04	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
4	18	+	5.00E+07	-	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
4	19	+	< LOQ	+	-	-	+	-	-	+	-	-	-	/	/	/	/	/	/
4	20	+	-	-	-	-	-	-	-	-	-	-	-	/	/	/	/	/	/
4	21	-	-	+	-	-	+	-	-	-	-	-	-	/	/	/	/	/	/
4	22	-	-	+	-	-	+	-	-	+	-	-	+	/	/	/	/	/	/
4	23	+	4.20E+04	+	-	-	+	-	-	+	D	D	D	/	/	/	/	/	/
4	24	-	-	+	-	-	+	-	-	+	D	D	D	/	/	/	/	/	/
4	25	+	< LOQ	-	+	1.80E+06	+	-	-	+	D	D	D	/	/	/	/	/	/
4	26	+	< LOQ	-	+	-	+	-	-	+	-	-	+	/	/	/	/	/	/
4	27	+	3.80E+05	-	+	< LOQ	+	-	-	+	-	-	-	/	/	/	/	/	/
4	28	+	2.10E+08	-	+	< LOQ	+	-	-	+	-	-	+	/	/	/	/	/	/
4	29	+	2.60E+08	-	+	< LOD	+	-	-	+	-	-	+	/	/	/	/	/	/
4	30	+	3.60E+04	+	+	1.90E+04	+	-	-	+	-	-	+	/	/	/	/	/	/

+: presence/positive; -: absence/negative; NA: not available; D: died; S: sold.

Supplementary Table 4. Determination of the LOD and the LOQ.

Replicate	Theoretical ssRNA transcript copies/ μ L															
	2×10^5		2×10^4		2×10^3		2×10^2		2×10^1		2×10^0		2×10^{-1}		2×10^{-2}	
	Cq	copies/ μ L	Cq	copies/ μ L	Cq	copies/ μ L	Cq	copies/ μ L	Cq	copies/ μ L	Cq	copies/ μ L	Cq	copies/ μ L	Cq	copies/ μ L
1	19.05	2.46E+05	23.09	1.56E+04	25.92	2.27E+03	29.78	1.63E+02	33.02	1.79E+01	37.05	1.14E+00	38.12	5.50E-01	ND	0.00E+00
2	19.04	2.48E+05	23.1	1.55E+04	26.09	2.02E+03	29.44	2.05E+02	33.32	1.46E+01	35.88	2.54E+00	39.76	1.80E-01	37.45	5.75E-01
3	19.51	1.80E+05	23.28	1.37E+04	25.76	2.53E+03	29.34	2.20E+02	33.76	1.08E+01	35.45	3.40E+00	37.78	6.94E-01	ND	0.00E+00
4	19.06	2.45E+05	23.18	1.47E+04	25.79	2.48E+03	29.51	1.96E+02	33.02	1.79E+01	35.65	2.97E+00	ND	0.00E+00	ND	0.00E+00
5	18.68	3.17E+05	23.27	1.38E+04	25.89	2.32E+03	29.54	1.92E+02	33.49	1.30E+01	35.98	2.37E+00	ND	0.00E+00	ND	0.00E+00
6	18.91	2.71E+05	23.28	1.37E+04	25.9	2.30E+03	29.22	2.39E+02	33.26	1.52E+01	37.05	1.14E+00	37.52	8.29E-01	ND	0.00E+00
7	19.02	2.51E+05	24.01	8.35E+03	25.65	2.73E+03	29.51	1.96E+02	32.59	2.40E+01	35.99	2.35E+00	37.54	8.18E-01	ND	0.00E+00
8	19.13	2.33E+05	23.22	1.43E+04	25.98	2.18E+03	29.09	2.61E+02	33.62	1.19E+01	35.51	3.27E+00	ND	0.00E+00	ND	0.00E+00
9	18.76	3.00E+05	23.23	1.42E+04	25.63	2.76E+03	29.37	2.15E+02	33.11	1.68E+01	35.25	3.90E+00	ND	0.00E+00	ND	0.00E+00
10	19.01	2.53E+05	23.26	1.39E+04	25.85	2.38E+03	29.2	2.42E+02	33.01	1.80E+01	35.93	2.45E+00	ND	0.00E+00	38.33	3.06E-01
mean	19.02	2.54E+05	23.29	1.38E+04	25.85	2.40E+03	29.4	2.13E+02	33.22	1.60E+01	35.97	2.55E+00	38.14	3.07E-01	37.89	8.81E-02
RSD%	1.18%	14.64%	1.12%	14.77%	0.55%	9.76%	0.68%	13.51%	1.04%	23.89%	1.72%	35.23%	2.45%	120.37%	1.64%	222.72%
No pos.	10/10		10/10		10/10		10/10		10/10		10/10		5/10		2/10	

ND= undetermined

Supplementary Table 5. Determination of intra-assay variation.

	Copies/ μ L		
	Sample1	Sample2	Sample3
Replicate 1	2.46E+05	7.67E+01	8.36E+03
Replicate 2	1.94E+05	7.68E+01	6.80E+03
Replicate 3	2.05E+05	9.06E+01	7.35E+03
mean	2.15E+05	8.14E+01	7.50E+03
RSD%	12.75%	9.83%	10.54%

Supplementary Table 6. Determination of inter-assay variation.

	Copies/ μ L		
	Sample 1	Sample 2	Sample 3
Run1	5.71E+04	7.15E+05	2.26E+03
Run 2	7.78E+04	8.08E+05	2.86E+03
Run 3	6.51E+04	7.53E+05	2.80E+03
Run 4	7.14E+04	8.55E+05	1.96E+03
mean	6.79E+04	7.83E+05	2.47E+03
RSD%	13.04%	7.85%	17.57%