Text S2: Extended Results

Study population characteristics

Among the 60 mothers, 52 were diagnosed with a SARS-CoV-2 PCR test in nasopharyngeal swabs while 8 mothers were seropositive (IgG positive). Most PCR tests (38/52, 73.1%) were performed as part of routine surveillance before labor. Table S1 specifies when SARS-CoV-2 was diagnosed either by PCR or by serology.

Validation of SARS-CoV-2 RNA extraction and detection methods in breast milk

To optimize SARS-CoV-2 viral RNA detection in breast milk samples, an initial analytical comparison aimed to determine the recovery of PEDV and MgV from spiked pre-pandemic breast milk samples using either a manual (MN) or an automated (Max) extraction method. Compared to spiked PBS, PEDV and MgV were recovered at 30 % (27-33 %) and 132 % (94-188 %) when extracted from whole milk samples with MN, respectively. Conversely, better recoveries (>100 %) were observed for both viruses extracted by Max. No significant inhibitions due the milk matrix were observed as depicted by the cycle threshold (Ct) values of 10-fold diluted RNA or RP gene reactions.

By spiking PEDV serial dilutions in milk samples, we further defined the detection ratios and limits of detection ($LoD_{95\%}$ and $LoD_{50\%}$), which are shown in Table S2. Results demonstrated similar sensibility of both extraction methods being 10 and 13 PEDV gc/100µL the limit of detection with 95 % confidence for MN and Max, respectively. These results suggest comparable analytical performances of MN and Max extraction methods for enveloped viruses.

Table S1: Type of COVID-19 diagnosis (PCR/serology) and average time to sample collection

COVID-19 diagnosis:	***n=60***
Positive nasopharyngeal PCR confirmation	n=52
confirmation 48 h before delivery	38
confirmation 48 h after delivery	2
confirmation > 48 h before delivery	9
confirmation > 48 h after delivery	3
Positive serology confirmation	n=8
confirmation 48 h before/after delivery	3
confirmation > 48 h before delivery	1
confirmation > 48 h after delivery	4
COVID-19 symptomatology	
Symptomatic prior or at enrollment	22
Average time from COVID-19 diagnosis to breast milk sample collection	
Days post-PCR confirmation: median	15.0
minimum and maximum	1 - 206
25th and 75th percentile	6.0 – 29.8
Days post-serology confirmation: median	15.5
minimum and maximum	1 - 226
25th and 75th percentile	(2.0 – 40.5)

Table S2. Concentrations, detection ratios, and limits of detection (LoD$_{95\%}$ and LoD$_{50\%}$) characterizing the analytical performances of a manual commercial kit (MN) and an automated assisted method (Max) to extract porcine epidemic diarrhoea virus (PEDV) spiked in breast milk

Levels of inoculated PEDV (gc/100uL)	MN				Max			
	Back calculated concentration (gc/100uL ± SD)	Detection ratio (+/total)	LoD$_{95}$ (gc/100uL)	LoD$_{50}$ (gc/100uL)	Back calculated concentration (gc/100uL ± SD)	Detection ratio (+/total)	LoD$_{95}$ (gc/100uL)	LoD$_{50}$ (gc/100uL)
10e3	1348.84 ± 152.84	6/6			3137.92 ± 756.22	6/6		
10e2	196.52 ± 10.10	6/6			575.86 ± 346.03	6/6		
10e1	25.82 ± 5.03	6/6	10.0	2.96	92.94 ± 44.48	6/6	13.38	3.10
10e0	2.05 ± 0.25	2/6			16.39 ± 4.45	3/6		
10e-1	-	0/6			-	0/6		