Supplementary information

Direct TaqMan Assay for the Detection and Genotyping of Bovine Viral Diarrhea Virus Type 1 and 2

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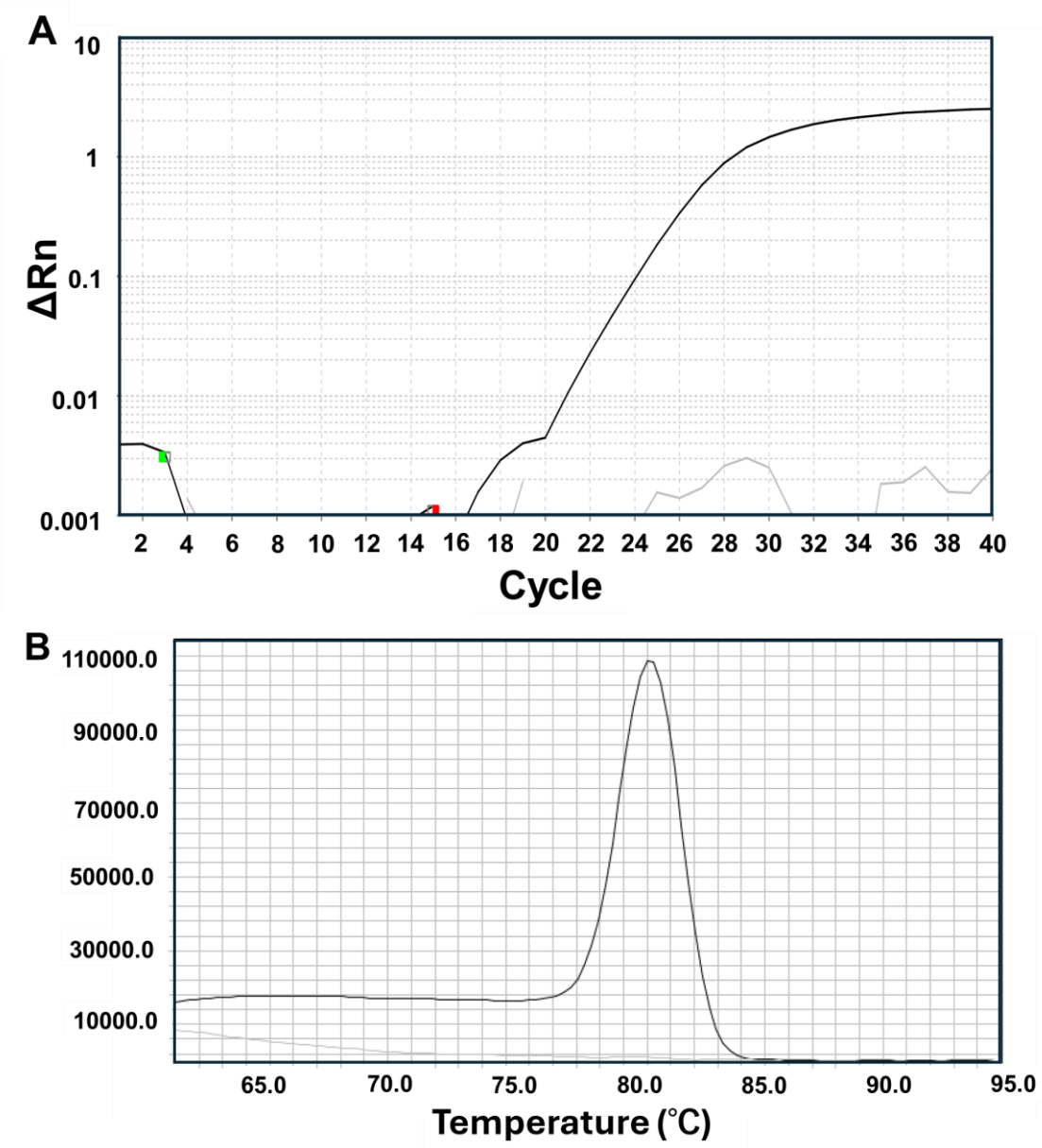

Fig. S1. Performance of the BVDV-specific primer set in the SYBR Green assay

BVDV-specific amplification using the SYBR Green assay is shown. BVDV positive and negative samples are indicated in black and gray, respectively. **(A)** The amplification plot of the SYBR Green assay. **(B)** The BVDV-positive sample showed a specific melting peak Tm at approximately 80 °C.

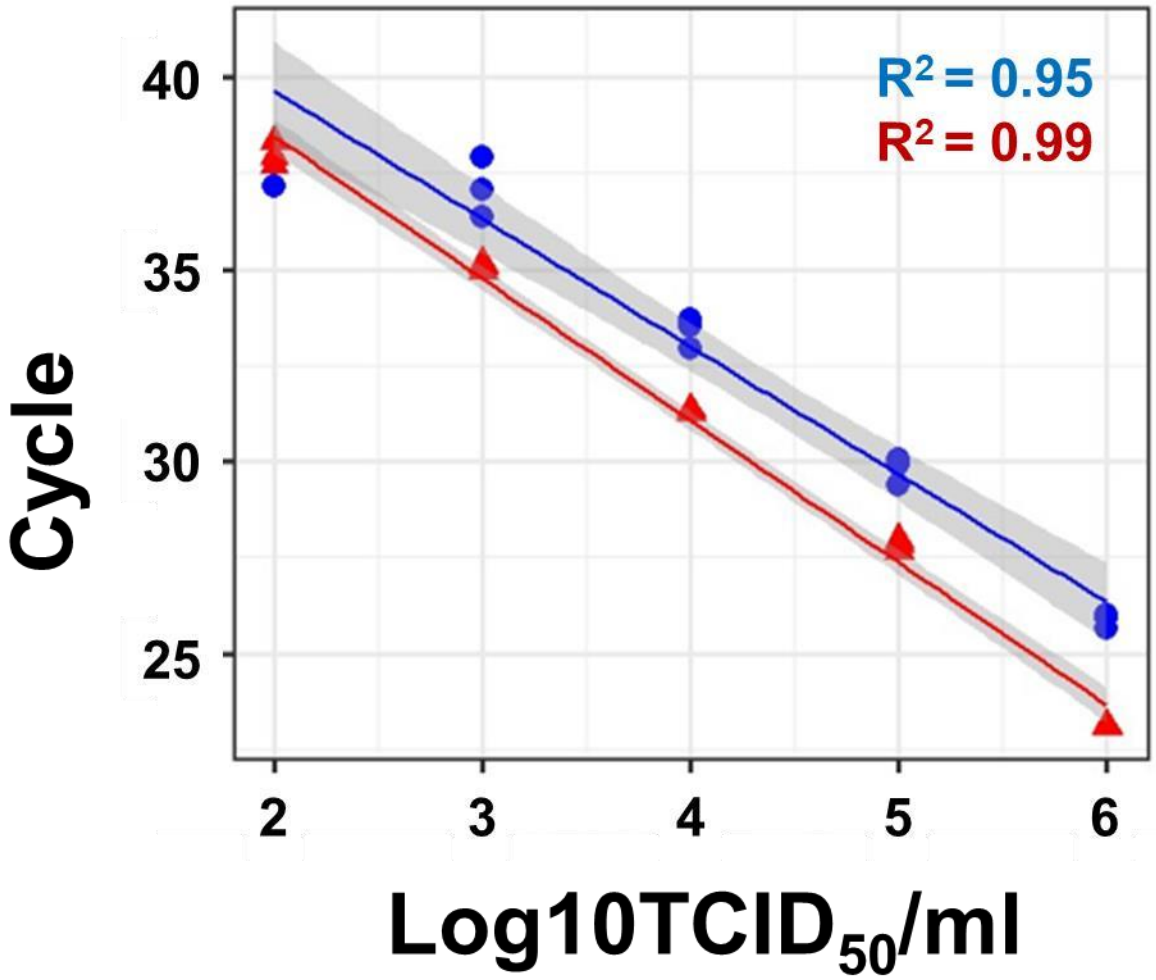

Fig. S2. Correlation between the viral titer and the TaqMan assay Ct value
The X axis indicates the log10 TCID50 and the Y axis indicates the Ct value. The blue plotted line shows BVDV1 values, and the red plotted line shows BVDV2 values. Each dot indicates the observed Ct in triplicates. The correlation between two variables is evaluated by linear regression analysis, as shown by the linear line.

Table S1. BVDV strain information obtained from the NCBI database to determine conserved regions.

Name of isolate	Subtype	Acc. No.	Name of isolate	Subtype	Acc. No.
Singer Arg	1a	DQ088995.2	CH-04-01b	1h	MW655625.1
Tokachi-22	1a	LC727647.1	SuwaCp	1k	KC853441.1
Tokachi-27	1a	LC727648.1	SD-15	1m	KR866116.1
1-NADL	1a	M31182.1	Shitara/02/06	1n	LC089876.1
CHN/HN	1a	ON901783.1	IS26/01ncp	1o	LC089875.1
Y2	1b	KY964311.1	camel 6	1q	KC695810.1
Tokachi-8	1b	LC727644.1	GBK_E+	2a	AB894423.1
Tokachi-13	1b	LC727645.1	SH2210-23	2a	HG426494.1
Tokachi-15	1b	LC727646.1	KZ-91-CP	2a	LC006970.1
Osloss	1b	M96687.1	Tokachi-47	2a	LC727649.1
Bega-like	1c	KF896608.1	Tokachi-31	2a	LC727650.1
10JJ-SKR	1d	KC757383.1	Olwein_*12	2a	MH231139.1
Isolate Carlito	1e	KP313732.1	Hokudai	2b	AB567658.1
SLO/1170/2000	1f	KX987157.1	Th/04_KhonKaen	3	FJ040215.1
UM/126/07	1h	LT631725.1	Italy-1/10-1	3	HQ231763.1

Table S2. List of comparison of different BVDV detection methods with Direct RT-qPCR

Name	PCR Steps	RNA extraction requirement	Genotyping BVDV	Reference
Ullah et al., 2024	1 step	No	Yes	Current study
Hoffmann B et al., 2006	1 step	Yes	No	[11]
Mari V et al., 2016	2 steps	Yes	Yes	[12]
Zhang J et al., 2022	1 step	Yes	No	[22]
Zhang YQ et al., 2015	1 step	Yes	Yes	[23]
Liang H et al., 2019	2 steps	Yes	No	[24]
Baxi M et al., 2006	1 step	Yes	Yes	[25]
Goto Y et al., 2021	2 steps	Yes	Yes	[26]