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Journal of Veterinary Medicine and Research

Research Article

Development of Real-Time Duplex RT-PCR for Thedetection of Bovine Viral Diarrhea Virus and Bovine Rotavirus in Cattle

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Submitted: 12 March 2015

Accepted: 26 April 2015

Published: 27 April 2015

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Keywords

- BVDV
- BRV
- Duplex real-time RT-PCR
- Detection
- Hydrolysis

Abstract

Bovine viral diarrhea virus (BVDV) and bovine rotavirus (BRV) are important pathogens of cattle. These pathogenselicitsigns that is sometimes difficult to differentiate. A real-time duplex RT-PCR assay would allow the simultaneous detection of the two viruses in the same sample. A real-time duplex RT-PCR assay was developed and optimized. The detection limit of the assay was 5 TCID50 or 100 RNA copies of BVDV and 20 TCID50 or 100 RNA copies for BRV. No cross reactivity with other bovine pathogens was detected. Both standard curves have amplification efficiency (E) of 1.06-0.95 and a correlation coefficient (R2) of 0.9917-0.9988, with the dynamic range of detection between 103 to 108copies per reaction. The sensitivity and specificityof this assay were compared with single real-time RT-PCR and conventional RT-PCR assays to verify its accuracy and sensitivity.The standardized duplex real-time RT-PCR confirmed the presence of viral RNA in 26 of 93 clinical samples. Two clinical samples were shown to be co-infected with both BVDV and BRV. This optimized real-time assay will be useful as a new sensitive diagnostic tool in epidemiological investigations of diarrheas in cattle.

ABBREVIATIONS

BVDV= Bovine viral diarrhea virus; BRV=Bovine Rotavirus;; RT-PCR=Reverse transcription-polymerase chain reaction; $TCID_{50=}$ tissue cultureinfected dose; FAM =Fluorescein amidite; HEX=Hexachloro-fluoresceine ; 5'-UTR: 5'= untranslated region; NCDV=Newcastle disease virus

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is a member of the *Pestivirus* genus of the family *Flaviviridae*. It is an important pathogen of dairy cattle [1]. It causes multiple clinical syndromes including acute diarrhea, mucosal disease, and fetal infection. Both horizontal and vertical transmission occurs, with a high attack rate and low mortality [2]. On the other hand, Bovine Rotavirus (BRV) is a double-stranded RNA virus, a member of the family *Reoviridae*. Rotavirus infections are the major cause of acute diarrhea among newborn animals and humans, as well as subclinical infection. Deaths occur as a consequence of dehydration [3].

These two major infections lead to economic losses that are detrimental to the farming industry in China [4]. Currently, diagnostic methods for these two bovine viral infections include virus isolation incell culture, immunoassay, serology and conventional reverse transcription PCR [5-10]. The aim of this study was to develop a rapid and sensitive real-time duplex-RT-PCR assay for simultaneous detection and differentiation of BVDV and BRV in cattle.

MATERIALS AND METHODS

Samples

The BVDV and BRVreference strains, field isolates and other non-diarrheal pathogens used in the study are listed in Table 1. All samples positive for BVDV and BRV as well as the negative were confirmed using ELISA (antigen detection)test kits(BVDV:IDEXX, USA; and BRV:BIOK, USA) and with conventional RT-PCR. Negative controls, included one bovine nasal swab and 1 bovine blood sample from healthy cattle and fecal samples collected at the Yangming Slaughterhouse, Nanning, China during pre-slaughter examinations.

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Table 1: Pathogens used.

Strain Virus/Genetic group	source	duplex Real-time RT-PCR result	
		BVDV/530	BRV/560
BVDV-1			
NADL	CVCC	+	-
AV68	CVCC	+	-
GX-4	GVRI	+	-
GX-0013	GVRI	+	-
GX-0443	GVRI	+	-
GX-7718	GXRI	+	-
GX-12	GXRI	+	-
BVDV-2			
GX-041	GVRI	+	-
GX-6136	GVRI	+	-
GX-1220	GVRI	+	-
GX-17	GVRI	+	-
GX-114	GVRI	+	-
Bovine rotavirus (BRV) NCDV	CVCC	-	17.32
BRV014	CVCC	-	+
NCDV	CVCC	-	+
GX-BRV-1	GVRI	-	+
GX-BRV-2	GVRI	-	+
GX-BRV-3	GVRI	-	+
GX-BRV-4	GVRI	-	+
GX-BRV-5	GVRI	-	+
GX-BRV-6	GVRI	-	+
GX-BRV-7	GVRI	-	+
GX-BRV-8	GVRI	-	+
Other pathogens			
Border Disease Virus(BDV)	GVRI	-	-
Mycobacterium bovis(MB)	GVRI	-	-
classical swine fever virus(CSFV)	GVRI	-	-
Infective bovine rhinotracheitis virus(IBRV)	CVCC	-	-
BovineCoronavirus (BC)	GVRI	-	-
Negative control			
Negative tissue sample			
Nasal mucus of normal bovine	SH	-	-
Blood of normal bovine	SH	-	-
Fecal negative sample			
A/Bos primigenius taurus / 1732	SH	-	-
A/ Bos primigenius taurus / 3363	SH	-	-
A/ Bos primigenius taurus /4523	SH	-	-
A/ Bos primigenius taurus /4462	SH	-	-
A Bos primigenius taurus /21	SH	-	-
A/ Bos primigenius taurus /12	SH	-	-
A/ Bos primigenius taurus /789	SH	-	-
A/ Bos primigenius taurus /35	SH	-	-
A/ Bos primigenius taurus /4520	SH	-	-
A/ Bos primigenius taurus /7	SH	-	-
A/ Bos primigenius taurus /3620	SH	-	-
A/ Bos primigenius taurus /49	SH	-	-
A/ Bos primigenius taurus /332	SH	-	-
A/ Bos primigenius taurus 28	SH	-	-
A/ Bos primigenius taurus 0137	SH	-	-
A/ Bos primigenius taurus /703	SH	-	-
A/ Bos primigenius taurus /46	SH	-	-
A/ Bos primigenius taurus /789	SH	-	-
A/ Bos primigenius taurus /45	SH	-	-
A/ Bos primigenius Taurus/ 733	SH	-	-
A/ Bos primigenius taurus /719	SH	-	-
A /Bos primigenius taurus /776	SH	-	-
A/ Bos primigenius taurus /782	SH	-	-
A/ Bos primigenius taurus /713	SH	-	-
A/ Bos primigenius taurus /708	SH	-	-
A/ Bos primigenius taurus /730	SH	-	-
A/ Bos primigenius taurus /744	SH	-	-
A Bos primigenius taurus /760	SH	-	-

CVCC: China veterinary culture collection center; GVRI: Guangxi Veterinary Research Institute; SH: Guangxi Provincial Bureau of Animal Health and Production slaughter house

Official review and Approval

The collection of these samples from the Yang ming Slaughter house could be considered as part of regular and routine examinations, therefore no official review and approval of the Guangxi Veterinary Research Institute was needed.

Extraction of viral RNA/DNA

The genomic viral RNA was extracted from 250 ul of infected culture supernatant by using the TRIZOL (Invitrogen, USA) in accordance with the manufacture's protocol, and viral and bacterial DNA used for evaluating the specificity of the new assay was extracted by using TIA Namp Genomic DNA kit (Tiangen, Beijing, China).The extracted RNA and DNA was re-suspended in distilled water and stored at -70 until used. cDNA was synthesized by random primers using the Revert Aid TM First Strand cDNA Synthesis Kit (Fermantas, USA), according to the manufacturer's protocol.

Primers and probes

Two pairs of primers and hydrolysis probes were designed and synthesized according to the conserved gene sequence of the BVDV 5' untranslated region (5'-UTR) and from the BRV VP6 gene [11-13]. The nucleotide sequences available on Gen Bank for BVDV1and 2 and BRV were aligned using DNAStart

MegAlign software, then primers and probes were design using the Primer Express software 2.0 according to the restrict design rules of real-time PCR primer and probes. A BLAST search was performed to verify oligonucleotide specificity. The BVDV-probe and BRV-probe, were labeled with FAM (emission wavelength of 518nm, the detection channel was 530) and HEX (554nm, 560) fluorophores at the 5'ends, respectively, and a nonfluoresent quencher BHQ1was attached at the 3'ends of each. These were compatible with a duplex format. Two sets of detection primers, which have been verified for the detection of BVDV and BRV, were used in this study. These primers and probes were synthesized and HPLC-purified by Invitrogen Inc. (Guangzhou, China). The details of the oligonucleotides for primers and probes are shown in Table 2.

RNA standard preparation

The synthesized DNA was amplified by a RT-PCR reaction kit (Tiangen, Beijing, China). The amplified DNA product was cloned into a pGM-T vector (TaKaRa, Dalian, China) according to the manufacturer's directions and was sequenced for accuracy. The recombinant plasmids pGM-T-VP6 and pGM-T-5'UTR were linearized using Not I restriction enzyme, gel purified, and used as a template with a Ribo Max T7 In-vitro Transcription System (Promega, Madison, Wisconsin, USA) according to the manufacturer's protocol. The RNA quantities were determined using UV spectrophotometry at 260 nm. Each concentration was converted to copy number of RNA moleculesasdescribed previously [14]. The standards of BVDV and BRV, each containing transcript RNA from10^8 to 1copies/μl, were prepared from thestock by 10-fold serial dilution in RNase-free H_2O. All RNA standards were stored at −70 till use.

Conventional RT-PCR

Conventional RT-PCR assay using the primers BVDV-F3/BVDV-F4 for BVDV1, 2 genotypes and BRV-F3/BRV-F4 for BRV (Table 2). BVDV-F3/BVDV-F4 are recommend by professional standard protocol of Entry-exit Inspection and Quarantine of China [15,16]. Briefly, the RT-PCR amplification was performed using RT-PCR master mix kit (QIAGEN Inc, Beijing, China), 1 μl (20 ng) of cDNA template and 50 pmol of each primer in a 25-μl reaction volume was used as per the manufacturer's protocol. The following thermal cycling times were used: initial cycle of 94˚C for 5 min, 35 cycles of 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s. After RT-PCR was performed, a 10-μl portion was analyzed using 1% agarose gel electrophoresis, and the amplified DNA product was visualized by ethidium bromide staining as described.

Real-time duplex RT-PCR

The real-time duplex RT-PCR reactions containing both primers and hydrolysis probes of BVDVand BRV were carried out using the Perfect Real Time RT-PCR Kit (TaKaRa, Dalian, China) according to the manufacturer's protocols. Real-time duplex PCR was performed using the Light Cycler 2.0 system (Roche, Molecular Biochemical, Mannheim Germany).The duplex RT-PCR reaction mixture contained 2×real time RT-PCR Premix (Perfect Real Time RT-PCR kit, TaKaRa, China), 0.2 μM primer of BVDV-F1 and BVDV-F2, 0.1μM probe of BVDV, 0.2μM primer of BRV-F1 and BRV-F2, 0.2μM probe of BRV, 1μl transcribedcDNA for clinical samples, for each positive control 1μl (20ng) of cDNAtemplate. Distilled H_2O was added to bring the final volume to 20 μL. The RT-PCR amplification consisted of 95˚C for 20 seconds (activation of Taq DNA polymerase), followed by 40 cycles of 95˚C for 15 seconds and 60˚C for 15 seconds. Fluorescence was measured at the end of each of 60˚C incubations. The entire duplex real-time RT-PCR amplification was performed for 1 h without post-RT-PCR handling.

Evaluation of real-time duplex RT-PCR

The sensitivity of the real-time duplex RT-PCR was determined by testing 10-fold serial dilutions of cell cultures infected with BVDV-CV24 ($10^{5.7}TCID_{50}$/mL), 10-fold serial dilutions of cell cultures infected with BRV-NCDV ($10^{4.3}TCID_{50}$/mL) and each RNA standards diluted serially 10-fold. [14,17].

Table 2: Primers and probes design for conventional RT-PCR and Real-time duplex RT-PCR assays.

Primers and Probes		Sequences(5'-3')	Positions[a] (bp)	Products (bp)
Real-time RT-PCR	BVDV-F1	TAGCCATGCCCTTAGTAGGACT	104-125	102
	BVDV-F2	GAACCACTGACGACTACCCTGT	184-205	
	BVDV-probe	FAM-CAGTGGTGAGTTCGTTGGATGGCT-BHQ1	148-171	
Conventional RT-PCR	BVDV-F3	AGGCTAGCCATGCCCTTAGT	100-119	244
	BVDV-F4	TCTGCAGCACCCTATCAGG	324-342	
Real-time RT-PCR	BRV-F1	TCATTTCAGTTGATGAGACCACC	984-916	112
	BRV-F2	ATTCAATTCTAAGCGTGAGTCCTAC	891-1005	
	BRV-probe	HEX-AATATGACACCAGCGGTAGCGGC-BHQ1	918-940	
Conventional RT-PCR	BRV-F3	GTATGGTATTGAATATACCAC	51-71	342
	BRV-F4	GATCCTGTTGGCCATCC	376-392	

[a]The positions correspond to genomes of BVDV Oregon CV24 strain(GenBank accession no.AF091605.1). The primers of BRV real-time PCR is derived form VP6 gene(GenBank accession no.AF317127.1), and the primers of conventional RT-PCR is derived from VP7 gene(GenBank accession no.M12394.1).

Table 3: Results of different concentrations of BVDV and BRV used in real-time duplex PCR assays.

	CT value			
RNA mixture of different concentration (BVDV/BRV) (copies/μL)	10^3 and 10^8	10^8 and 10^3	10^7 and 10^5	10^5 and 10^7
BVDV-specific single real-time PCR at 530 channel	30.34	13.47	16.82	23.59
Real-time duplex RT-PCR at 530 channel	29.71	13.50	16.65	23.54
BRV-specific single real-time PCR at 560 channel	9.79	26.53	19.87	13.19
Real-time duplex RT-PCR at 560 channel	9.51	26.74	20.69	12.78

Table 4: The results of real-time duplex PCR of clinical sample.

sample	Origin/source	real-time duplex RT-PCR		conventional RT-PCR	
		BVDV	BRV	BVDV	BRV
Sample 1	Holstein cow/Guangxi/NN	+	-	+	-
Sample 2	Holstein cow/Guangxi/NN	+	-	+	-
Sample 3	Holstein cow/Guangxi/NN	+	-	+	-
Sample 4	Holstein cow/Guangxi/NN	+	-	-[a]	-
Sample 5	Holstein cow/Guangxi/NN	+	-	+	-
Sample 6	Holstein cow/Guangxi/NN	+	-	+	-
Sample7	Holstein cow/Guangxi/GL	+	+	+	+
Sample 8	Holstein cow/Guangxi/ GL	+	-	+	-
Sample 9	Holstein cow/Guangxi/ GL	+	+	+	+
Sample 10	Holstein cow/Guangxi/ GL	+	-	+	-
Sample 11	Holstein cow/Guangxi/ GL	+	-	+	-
Sample 12	Holstein cow/Guangxi/LZ	+	-	+	-
Sample 13	Holstein cow/Guangxi/LZ	+	-	+	-
Sample 14	Holstein cow/Guangxi/ LZ	+	-	+	-
Sample 15	Holstein cow/Guangxi/ LZ	+	-	+	-
Sample 16	Holstein cow/Guangxi/ LZ	+	-	+	-
Sample 17	Holstein cow/Guangxi/ LZ	+	-	-[a]	-
Sample 18	Holstein cow/Guangxi/ LZ	-	-	-	-
Sample 19	Holstein cow/Guangxi/ LZ	-	+	-	+
Sample 20	Holstein cow/Guangxi/NN	-	+	-	-[a]
Sample 30	Holstein cow/Guangxi/NN	-	+	-	+
Sample 31	Holstein cow/Guangxi/NN	-	+	-	+
Sample 32	Holstein cow/Guangxi/ LZ	-	+	-	+
Sample 33	Holstein cow/Guangxi/ LZ	-	+	-	+
Sample 34	Holstein cow/Guangxi/ LZ	-	+	-	+

NN: Jinguang diary farm, Nanning, Guanxi province; LZ: Zhegucun diary farm, Nanning, Guanxi province; GL: fushui diary farm, Guilin, Guanxi province
[a]false negative samples determined by sequence analysis.

To determine the linearity of the reaction .Ct values were obtainedafter amplification andwere plotted against RNA copies. The standard curve was generated by plotting the Ct values versus $\log_{10}$RNA transcript copies of BDVD and BRV, respectively.

To evaluate its analytical specificity, the real-time duplex RT-PCR test was performed on a panel of viral strains (Table 1). The known 28 fecal samples, one nasal mucus sample, and one blood sample were collected from normal cattle at the slaughterhouse. These cattle were asymptomatic and tested negative for BVDV/BRV by antibody test (BVDV: IDEXX ELISA; BRV: RIOK ELISA). Four strains of BVDV, two strains of BRV, eight isolates of BRV and four different bovine DNA and RNA viruses were used to test the specificity of the Real-time duplex RT-PCR assay.

The intra-assay reproducibility was evaluated by testing five replicates of five different RNA titers of BVDV-CV24, GX-014,or BRV-NCDV, and the inter-assay reproducibility were determined with five different titers of RNA from each virus on three separated days.

Various concentrations of RNA transcript containing BVDV-CV24 and BRV-NCDV were mixed together, and tested by real-time duplex RT-PCR and single real-time RT-PCR to assess the

interference between high and low concentration templates, as described [18].

Testing of clinical samples

93 fecal swab samples were collected randomly from cattle with diarrhea from various dairy farms (as shown in Table 4) in the Guangxi province during 2013. A written informed consent was obtained from each participating farm owner for the taking of fecal swab samples. The veterinarians of the participating farms collected fecal swab samples from the calves, and the fecal samples were considered as part of regular and routine clinical-diagnostic care. No official review and approval of the animal protocol by Guangxi Veterinary Research Institute was needed. 92 clinical samples were tested including samples from 32 calves that were less than 6 month old, 41 calves/cow from 6 months to 3 years old and 20 heifers over 3 years old. The swab samples were placed into 1ml sterile water, and then extraction of RNA was processed as described above. The concentration range of RNA was from 103 ng to 620ng. Real-time duplex RT-PCR assay and conventional RT-PCR were performed as describe above to assess the reliability of the methods for the rapid detection of clinical samples. The positive amplified amplicon products were cloned into PMD-18T vector (TaKaRa, Dalian, China) and sequenced to verify for the false positive amplifications.

RESULTS AND DISCUSSION

Sensitivity of the real-time duplex RT-PCR

By using 10-fold serial dilutions of cell culture infected with BVDV-CV24 or BRV-NCDV, the detection limits of the real-time duplex RT-PCR were determined to be 5 $TCID_{50}$ for BVDV and 20 $TCID_{50}$ for BRV. Using the RNA transcribed from the plasmids as standards, the detection limits of the real-time duplex RT-PCR were determined to be 100copies/μl for both of the viruses (Figure 1A and 1B), whereas the conventional RT-PCR could only detect 10^5 copies/μl for BVDV and 10^4 copies/μl for BRV(date not show). Real-time duplex RT-PCR was about 100-fold higher insensitivity than conventional RT-PCR assay. To define the cut-off value, 30 negative samples were tested using real-time duplex RT-PCR to calculate the mean of CT cycles (x) and the standard deviation (SD) among negative samples. The cut-off valuewas defined by a formula: cut-off value = x ±3SD. The cut-off CT value for positive and negative results was determined as 35 cycles. Tested samples with CT values below 35 were considered as positive, and vice versa for negative samples.

Standard curve

The standard curves (CT value vs. log10 RNA copies) of the real-time duplex RT-PCR and single real-time RT-PCR were linear with a dynamic range of detection between 10^8 to 10^3 copiesof in-vitro transcribed RNA per reaction for standards of BVDV and BRV (Figure 2A and B). The amplification efficiency was not markedly affected in the real-time duplex assay compared to the single assay.

Specificity of the real-time duplex RT-PCR

The real-time duplex RT-PCR for BVDV, using spectrometer channel 530, detected the2 reference strains of BVDV1 and 5 field isolates of BVDV1. Also, it detected 5 strains of BVDV. The real-time duplex RT-PCR for BRV, using spectrometer channel 560, detected 2 reference strains, and eightfield isolates of BRV. Real-time duplex RT-PCR didn't detect the otherpathogens (Table 1).

Reproducibility of the real-time duplex RT-PCR

The coefficients of variation (CVs) of CT values for cell cultures infected with CV24, NADL, GX-041, NCDV and BRV014 were carried out in five replicates. The intra-assay CVs for CT values ranged from 0.96% and 2.71%, and the CVs of the inter-assay ranged between 0.89% and 1.41%, indicating the reproducibility of the new assay (data not shown).

Interference test of the real-time duplex RT-PCR

As shown in Table 3, there was no difference in amplification efficiency of BVDV and BRV between the singlereal-time RT-PCR and real-time duplex RT-PCR formats, as measured by the slopes of amplification curves during the exponential phase and the CT values obtained with individual samples.Although there were some variationsin the amplification curves, when comparing

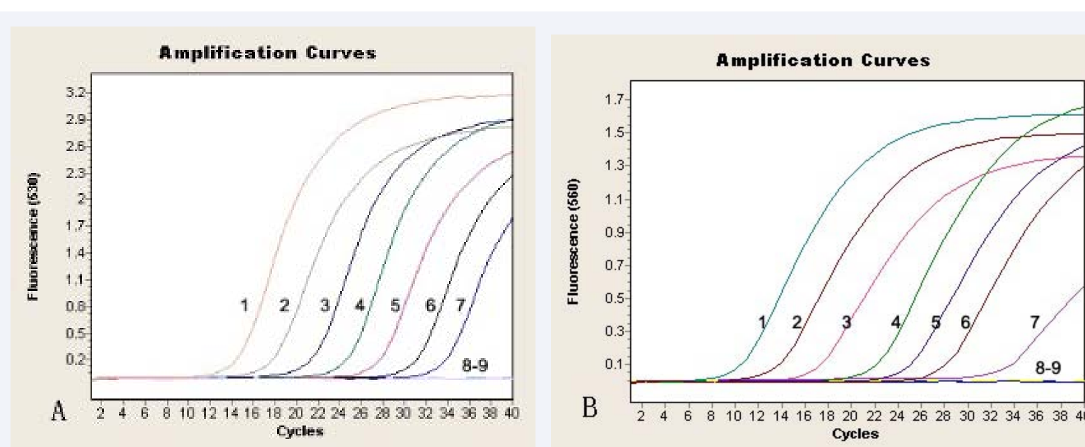

Figure 1 The detection limit of real-time duplex RT-PCR for BVDV and BRV. Samples are diluted serially 10-fold RNA transcript for BVDV CV24 and BRV NCDV. (A) Result of BVDV; (B) Result of BRV, 1: 108 copies/μl,2: 107 copies/μl,3: 106 copies/μl,4: 105 copies/μl,5: 104 copies/μl,6: 103 copies/μl,7: 102 copies/μl,8: 101 copies/μl,9 : 100 copies/μl.

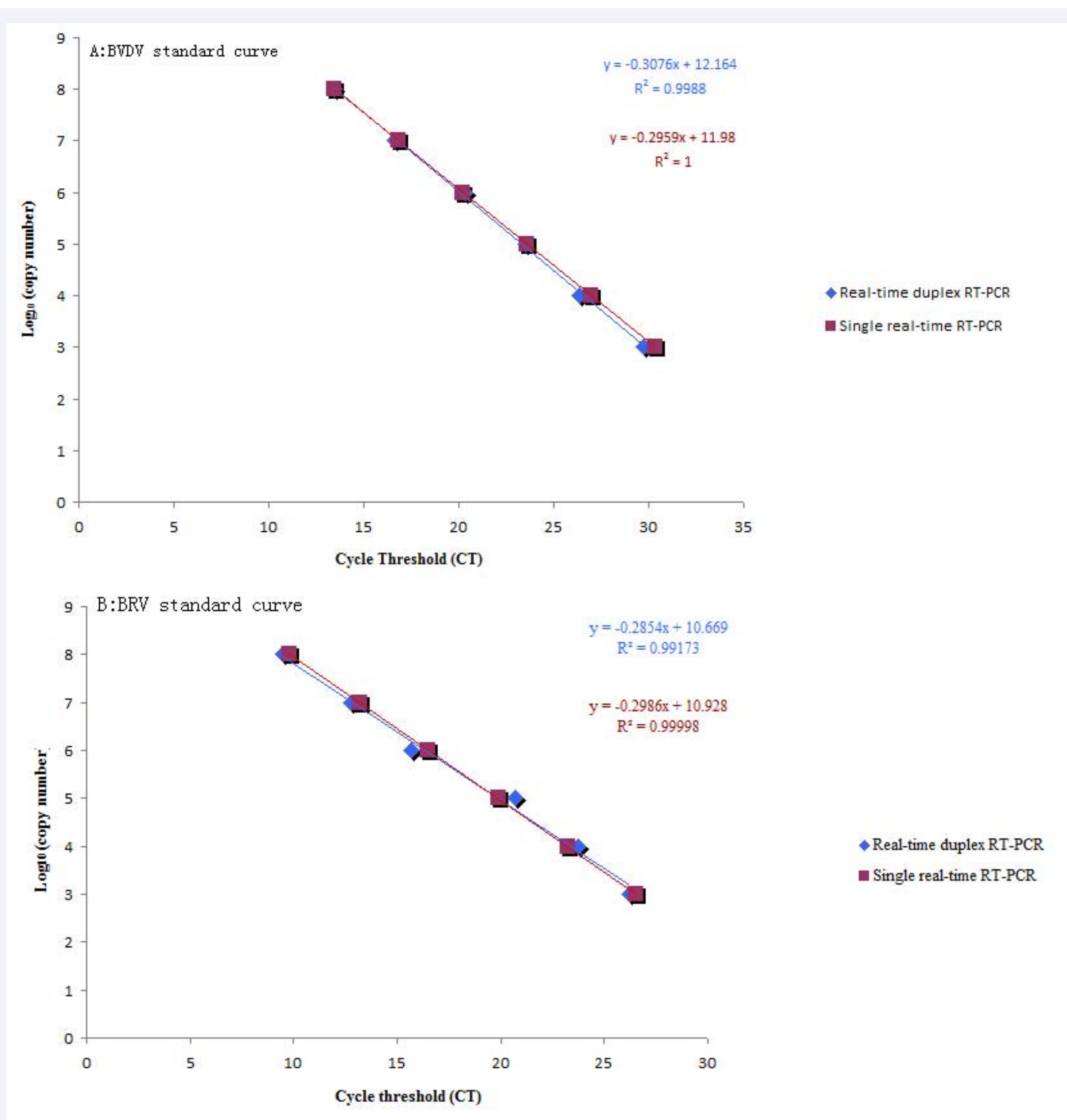

Figure 2 Standard curves and dynamic ranges of real-time duplex PCR. 10-fold dilution series ranging from 10^8 to 10^3 copiesof in vitro transcribed RNA per reaction was tested. Each plot represents amplifications of each dilution. The x-axis indicates Ct values. The y-axis indicates the virus concentration expressed in $\log_{10}$copies/reaction. The examplesshow standard curves of the amplification of BVDV **(A)** and BRV **(B).** A amplification efficiency (E) of 1.06 for BVDV and 0.95 for BRV, respectively, was estimated using the standard curve slope as indicated by the formula E= [10(-1/slope)-1]× 100%. Formula y1 and y2 express the linearity of single real-time RT-PCR and real-time duplex RT-PCR respectively.

the duplex assay with the single-target assays, the detection levels were not affected. The results suggest that variable viral concentrations do not result in significant differences in amplification performance.

Clinical evaluation

To assess the sensitivity of the real-time duplex RT-PCR, we compared the assay with conventional RT-PCR. The results are shown in Table 4. Twenty-six fecal samples were positive by both real-time duplex RT-PCR and conventional RT-PCR assay, and the results were verified by sequence analysis. Two cases of co-infection by the BVDV and BRV were identified with the real-time duplex RT-PCR. Based on the standard reference curve developed for BVDV and BRV (Figure 2 A,B). These CT values of two BVDV discordant samples correspond to viral loads are $10^{3.14}$ and $10^{4.08}$ copies/µl, and the one BRV discordant samples had $10^{3.63}$ copies/µl. These values were below the detection limits of BVDV (10^5) and BRV (10^4) of conventional RT-PCR

The results of age range of the BVDV and BRV positive cattle were as followed: the detection rate of BVDV was 31.7% (13/41) for animals between 6 to 36 months, and for BRV positive cattle the detection rate of 25 % (8/32) was seen in animals less than six months. No BRV positive was detected from the group of over 36 months of age.

Although BVDV and BRV infections are considered common events in cattle, limited data exist on the prevalence of these infections. This lack of knowledge could be attributable in part to an absence of suitable diagnostic techniques for these infections.

Virus isolation cannot be considered a valid diagnostic tool due to the poor replication of these viruses in the tissue cultures.

The aim of this study was to develop a rapid and sensitive real-time duplex RT-PCR assay for simultaneous detection of BVDV and BRV with high specificity, sensitivity, and reproducibility. Hydrolysis probes showed excellent assay specificity, and no cross-reactivity was observed with other bovine pathogens. The minimum detection levels of the in-vitro transcripts that were consistently detected by the real-time duplex RT-PCR were 100 RNA copies of BVDV and BVDV both. The R^2 values of all of the standard curves were larger than 0.99, and the amplification efficiency was between 0.95 and 1.06, which meets the requirement for fluorescence quantitative RT-PCR standard curve. The assay is also timesaving and capable of extremely rapid and accurate diagnosis of both viruses within 1h.

Another important advantage of this real-time duplex RT-PCR is recognition of co-infections, as described for other animal viral pathogens [19-21]. BVDV and BRV exhibit similar clinical signs in cows co-infected with these pathogens. Of the 93 field samples, two were co-infected with BVDV and BRV, in these young animals under 6 months of age. These 2 co-infected had diarrhea, high fever, in appetence and dehydration, and the duration of the diarrhea was more than 1 month. One died from the diarrhea (Table 4). This may be because co-infections aggravated one another [22]. There is a need for epidemiological surveillance of clinically normal cattle less than 6 months. Simultaneous detection and differentiation of these two viruses will be beneficial in disease control in cattle herds in China.

BVDV is a positive-sense, single stranded RNA virus with a genome size of approximately 12.5 kb. There are two major genotypes of BVDV1 and BVDV2 based on the genetic relatedness, and the 5'UTR are the most conserved region and there for esuitable for targets for reverse transcription polymerase chain reaction (RT-PCR) assays [23-25]. All 17 BVDV-positive samples those were confirmedby real-time RT-PCR were clustered within the BVDV1 genotype, as revealed by DNA sequence analysis of the 102 bp RT-PCR product. The real-time duplex -PCR method will be particularly useful in detecting BVDV from cattle herds in China.

CONCLUSION

The real-time duplex RT-PCR assay described in the present study meets the current need for rapid and sensitive detection of BVDV and BRV in cases of severe diarrheal disease of unknown etiology. This assay will be useful for the surveillance in the Chinese cattle population.

ACKNOWLEDGEMENTS

This research was supported by Guangxi Natural Science Foundation, code 2011GXNSFA018096 and Guangxi Government Senior Scientist Foundation, code 2011B020. Authors sincerely thank Dr. Herbert Vankruiningen for his critical review and correction of the manuscript.

Conflict of Interest

All authors declare that they have no conflict or financial interests.

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Cite this article

Xie Z, Fan Q, Xie Z, Pang Y, Deng X, et al. (2015) Development of Real-Time Duplex RT-PCR for Thedetection of Bovine Viral Diarrhea Virus and Bovine Rotavirus in Cattle. J Vet Med Res 2(2): 1020.