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Short communication

Genetic diversity of bovine viral diarrhea viruses in commercial bovine serum batches of Chinese origin

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ABSTRACT

Bovine viral diarrhea virus (BVDV) is often detected in commercial bovine serum. BVDV genetic diversity was investigated in commercial bovine serum of Chinese origin. Twenty-two batches of bovine serum were obtained from 10 suppliers with different geographic origins in China, and 20 batches of bovine serum were positive by reverse-transcription polymerase chain reaction (RT-PCR) and sequencing. Phylogenetic reconstructions of partial 5′UTR sequences indicated that the samples examined in this work clustered within the BVDV type 1 and BVDV type 2 genotypes. Interestingly, 3 sample sequences clustered into CSFV. These results suggest a high genetic diversity in Chinese BVDV field isolates. This study will benefit epidemiological surveys of BVDV detected in China.

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Bovine viral diarrhea virus (BVDV), the etiological agent of bovine viral diarrhea/mucosal disease, is a worldwide pathogen in cattle that causes significant economic losses to agriculture (Xue et al., 2011). BVDV, together with classical swine fever virus (CSFV) and sheep border disease virus (BDV), belongs to the genus Pestivirus in the Flaviviridae family (Byers et al., 2009). Recently, atypical bovine pestiviruses such as Th/04_KhonKaen virus, D32/00-'HoBi' and CHKaHo/cont have been referred to as BVDV-3 (Liu et al., 2009a). Bovine viral diarrhea virus infection was first reported in Olafson et al. (1946). Subsequently, BVDV was found to be epidemic worldwide in cattle farms. In the intervening 66 years, many important advances have been made in understanding this virus and the disease it produces. However, in China, progress with BVDV has been limited. To date, no commercial BVDV vaccines are available on the market, and no management systems or control programs have been conducted in China. The genetic diversity of BVDV needs to be considered when designing and constructing effective vaccination strategies for the virus, and a vaccine must accurately reflect the genotypes and antigenic types present in the country of use (Mahony et al., 2005). The genetic and antigenic diversity of BVDV are not well known in China. Bovine serum is an important material in medicine and biological products. However, bovine serum is often contaminated by bovine viral diarrhea virus (Bolin and Ridpath, 1998). Recently, atypical bovine pestiviruses were detected in many commercial fetal bovine serum samples from different geographic origins (Xia et al., 2011). Every batch of commercially available bovine serum is a mixture of collected raw serum material from different cattle farms near the supplier. Therefore, commercial bovine serum is a good material for investigating BVDV genetic diversity. The purpose of this work was to determine the genotypes of BVDV detected in commercial bovine serum in China.

A total of 22 batches of bovine serum were purchased from 10 commercial suppliers. The 22 batches of bovine serum came from 8 provinces in China (Table 1). Total RNA was extracted from serum samples using TRIZOL (Invitrogen, China) according to the manufacturer's instructions. The extracted RNA was reverse-transcribed using the M-MLV Reverse Transcriptase Kit (Invitrogen, USA) as specified by the manufacturer. The 5′ UTR primers were selected as described previously (Ridpath and Bolin, 1998). The 5′ UTR genomic region provides meaningful inferences as this region has the highest degree of sequence conservation and is effectively amplified by RT-PCR (Vilcek et al., 2001). The 5′ UTR primer sequences were as follows: sense primer: 5′ CAT GCC CAT AGT AGG AC 3′; antisense primer: 5′ CCA TGT GCC ATGTAC AG 3′, and the primers are not reliable for amplifying HoBi-like viruses. Similar 280 bp fragments of the 5′ UTR region were amplified from 20 batches of bovine serum, and the amplified fragments were purified and cloned into a pMD18-T vector (TaKaRa, Japan), and 3 clones of every sample were sequenced. Sixty sequences from 20 batches of bovine serum were found, after sequence alignment,

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Table 1
The batches of bovine serum tested by RT-PCR.

Origin	Supplier	Sample ID		BVDV-1	BVDV2	CSFV
NMG	A	1	S11, S12, S13	1 m(S11[10], S12, S13[9])		
		4	S41, S42, S43	1 m(S41, S42[9], S43[3])		
JL	B	2	S21, S22, S23	1 m(S22, S21, S23[10])		
		3	S31, S32, S33	1 m(S31[3]) 1b(S32[8], S33[8])		
	G	15	S151, S152, S153	1a(S151[6], S152), 1p(S153[4])		
		16	S161, S162, S163	1 m(S161[9]), 1b(S162[7], S163[8])		
TJ	C	7	S71, S72, S73	1 m(S71, S72, S73[12])		
		8	S81, S82, S83	1 m(S81[11], S82[11]), 1q(S83[2])		
	D	9	S91, S92, S93	1a(S91[6], S92[6]), 1b(S93[7])		
		10	S101, S102, S103	1 m(S101, S102), 1b(S103[7])		
SD	E	5	S51, S52, S53	1 m(S52), 1q(S53[1])	S51[13]	
		6	S61, S62, S63	1 m(S62[10], S63[10]), 1q(S61[1])		
GS	F	12	S121, S122, S123	1 m(S121, S122, S123)		
		13	S131, S132, S133	1 m(S133), 1q (S131[1], S132[1])		
HN	H	17	S171, S172, S173		S172[13]	S171,S173[14]
		19	S191, S192, S193	1 m(S193[9]), 1q (S191[2], S192[2])		
HLJ	I	14	S141, S142, S143	1 m(S141[3]), 1c(S142[5])	S143	
		18	S181, S182, S183	1e(S182), 1c(S183[5]), 1a(S181)		
JS	J	11	S111, S112, S113	1 m(S111[12], S113[12])		S112[14]
		20	S201, S202, S203	1a(S201, S202), 1p(S203[4])		

Superscript: the same number means consensus sequence.

the consensus sequences were eliminated, thirty-four different sequences from 20 batches of bovine serum were found, containing at least one species of pestiviruses. The 34 newly determined 5′ UTR sequences were deposited in GenBank with the accession numbers KF006955–KF006975 and KJ690679–KJ690691.

The BVDV 5′ UTR of the samples were aligned with the corresponding regions of the pestivirus reference sequences retrieved from GenBank using the Clustal W program in MegAlign of Lasergene 7.2 software (DNASTAR Inc. Madison, WI, USA). Then, phylogenetic trees were constructed based on the 251 nucleotide region of the 5′ UTR. Bootstrap values were based on 1000 replicates using the neighbor-joining method (Kimura two-parameter method) with Molecular Evolutionary Genetics Analysis (MEGA version 4.1) software. Phylogenetic analysis indicated that the sequences belonging to BVDV-1 should be further classified into 8 subgenotypes: BVDV-1 m (29/60), BVDV-1b (6/60), BVDV-1a (7/60), BVDV-1c (2/60), BVDV-1p (2/60), BVDV-1e (1/60) and BVDV-1q (7/60). Of these sequences, BVDV-1 m was the most frequently identified, strain ZM-95 was the first isolated BVDV-1 m in China (Xu et al., 2006). Of these sequences, 29 sequences from 15 batches of bovine serum belonged to BVDV-1 m; the percentage of BVDV-1 m was 48.33% (29/60), and nucleotide homology among these sequences was in the range 91.1–100%. BVDV-1b is considered to be a predominant BVDV-1 strain circulating in Chinese cattle. In this study, 6 sequences from 4 batches of bovine serum belonged to BVDV-1b, with the percentage being 10% (6/60). Based on this result and a previous report (Xue et al., 2010), the percentage of BVDV-1 m identification was higher than that of BVDV-1b. Hence, BVDV-1 m is considered to be more predominant than BVDV-1b in Chinese cattle population. BVDV-1p and BVDV-1c were found in 2 sequences from 2 batches of bovine serum. BVDV-1a is the usual subtype of BVDV-1 vaccines, which is dominant in the UK and widely distributed in USA. Likewise, the BVDV isolates predominating in a neighboring country, namely Korean (Booth et al., 2013; Oem et al., 2009). BVDV-1a has been found in Chinese pig herds but has been unique reported in the Chinese cattle population. In this study, BVDV-1a was found in 7 sequences from 4 batches of bovine serum. BVDV-1q is a newly identified subgenotype from cattle, pigs and camels in China (Deng et al., 2012; Gao et al., 2013; Gong et al., 2013). In this study, there are 7 sequences from 5 batches of bovine serum that belonged to BVDV-1q. BVDV1e was isolated in Italian Austria and UK (Booth et al., 2013). It had not yet been found in China; however, in this study, only one sequence was found in 1 batch of bovine serum. BVDV-1 h and BVDV-1f are the predominant BVDV1 strain prevalent in Australia (Mahony et al., 2005), but it was not detected in this work (see Fig. 1).

To date, BVDV-2 has been grouped into at least four BVDV-2 subtypes (BVDV-2a–BVDV-2d) (Flores et al., 2002; Mishra et al., 2008; Vilcek et al., 2004).A BVDV-2 strain, SD-06, was first isolated from cattle in China (Zhu et al., 2009b). Subsequently, many BVDV-2 strains were isolated from cattle and pigs in China (Liu et al., 2012; Tao et al., 2013; Zhu et al., 2009a), which belonged to BVDV-2a. Compared with the endemic situation of BVDV-1, the prevalence of BVDV-2 infection in cattle is seemingly much lower in China. In this study, 3 sequences from 3 batches of bovine serum belonged to BVDV-2, and phylogenetic analysis showed that they formed a distinct branch from other previously reported isolates. The sequences belong to BVDV-2b, with a 93.7–95% nucleotide sequence homology to BVDV-2b reference strain VS-123.4 and 34b (Flores et al., 2002).

Bovine viral diarrhea virus, classical swine fever virus and border disease virus are all members of the genus pestivirus. However, in contrast with the wide host range of BVDV, CSFV only infects pig and wild boars. In this study, phylogenetic analysis showed that 3 sequences from 2 batches of bovine serum belonged to CSFV. This result is in agreement with a previous observation that BVDV strain HEN03 (KC176778) was found in cattle blood from Henan province. The Npro and E2 genomic region are important to the pestivirus genotype, attempt to amplify these genes and phylogenetic analysis. It is possible that the storage conditions and time of the collection of these samples were poor, these genes could not been amplified in many of these samples, although we did try many times (data not shown). Early in 1957, Yuan Qingzhi, the inventor of the Chinese hog cholera lapinized vaccine strain, his studies have shown that the vaccine strain can be infected and breeding in cow, and the Cattle Organization vaccine of the Chinese hog cholera lapinized vaccine strain were produced and

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