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Research Article

Assessment of Hygiene Habits during the COVID-19 Pandemic in Students: A Preliminary Study

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Abstract

Bovine viral diarrhea virus 3 (BVDV-3), also designated as Hobi-like Pestivirus or atypical ruminant Pestivirus, causes the similar clinical presentation in cattle following infection with BVDV. Natural infection in cattle and contaminated in FBS with BVDV-3 and increased demand for FBS led to widespread distribution of FBS originating in BVDV-3 endemic regions, even in the world. In present short communication, we reported the condition of BVDV contamination in FBS and poorly growing cells. The results indicated that a commercial FBS batch designated as C1 was determined as BVDV-3. And the others FBS or cells with BVDV nucleic acid positive was confirmed to be BVDV-1. The detection and contaminant elimination of BVDV in the commercial biological products are critical and benefit for the cattle industry and even in the science research.

INTRODUCTION

Bovine viral diarrhea virus 3 (BVDV-3), was also named atypical bovine pestiviruses, but still no defined subtypes [1,2]. BVDV-3 was also designated as atypical ruminant Pestivirus, Hobi-like Pestivirus, or Pestivirus H. The clinical presentations infected BVDV-3 in cattle has similar BVDV-related symptoms, mostly subclinical and mild clinical symptoms, occasional hemorrhagic syndrome, respiratory disease, and reproductive loss. BVDV-3 clinical infection poses difficulties for BVDV eradication programs. Decaro et al. developed a nested PCR devoting for detection of viruses infecting cattle [3]. It helped to ensure the live vaccines and other biological products without pollution. It does as well as evaluates the molecular epidemiology of BVDV-3. Based on 5'UTR, complete N(pro), and E2 gene regions, the pestiviruses was proposed classified into nine species: BVDV-1~BVDV-3, classical swine fever virus (CSFV), Border disease virus (BDV), Tunisian sheep virus (TSV), Pestivirus of giraffe, and Antelope and Bungowannah [4]. According reference report, BVDV-3 shared nucleotide sequence identities of less than 70 % with other BVDV [5].

The emergence of BVDV-3 in cattle around the world especially emphasized and posed the challenge to the disease control measures. These solutions include developing vaccines with improved cross-protective potential and enhancing disease diagnostics [6]. BVDV-3 was firstly isolated from FBS in Brazil [7]. It is also reported that bovine had natural infection in Southeast Asia and Europe. BVDV-3 is presumed to be circulating among cattle in these countries. Because of the increased demand for Foetal Calf Serum (FBS), the pathogen spread more widely in BVDV-3 contaminated areas. The contaminated FBS with BVDV-3 can be very dangerous for the spread of disease in the surrounding area. Partial 5' untranslated region (5'UTR), have been a pivotal sequence in phylogenetic analyses of BVDV isolates. In present study, BVDV-3 and BVDV-1 was identified in contaminated commercial FBS in China.

MATERIALS AND METHODS

In order to find out the cause of cellular abnormality in the cell culture process of our laboratory, we tested the FBS used in our lab and cell lines we cultured by general primers of BVDV. We used a pair of primers reported in previous study for BVDV detection [8]. The FBS batches were all from commercial company. Here, we assigned C. G. and J company. The cell lines here mentioned were DF1, Marc-145, MDBK, BT, M-104, BHK, and M-007. The PBS, equine serum and trypsin we used also were detected. The total RNA of suspicious FBS and cells and other samples were extracted with RNA Extraction Kit (TaKaRa, China). following the manufacturer's instructions. Viral cDNA was obtained from RNA with reverse transcriptase (M-MLV, Invitrogen). Then a DNA fragment length 288bp targeting BVDV 5'UTR was obtained by the polymerase chain reaction (PCR),

amplification with a pair of detection primers BVP1 and BVP2, the primer sequences were 5'-TAGCCATGCCCTTAGTAGGAC-3' and 5'-CTCCATGTGCCATGTACAGCA-3' [8]. The PCR program was 35 cycles: 94°C denaturation for 30 s, 57°C annealing for 30 s, and 72°C elongating for 30 s. Finally, 72°C elongating additional 10 min, following cooled samples at 4°C. Next, the 5 μL PCR products were analyzed by nucleic acid electrophoresis on 2% agarose gel at 140 V for 15 min.

The amplicons from three commercial FBS and a cell line Marc-145 were purified with an gel extraction kit (TIANGEN, China) and sequenced in biotechnology company. We assembled the sequenced nucleotide sequences and analyzed them with DNASTAR software: SeqMan program of Lasergene. Five sequences obtained from FBS and cultured cells in present study and 38 reference sequences downloaded from the NCBI GenBank database were compared. We constructed the phylogenetic tree by the Maximum Likelihood method with MEGA7.0.

RESULTS AND DISCUSSION

For investigating the BVDV infection in serum and cells in our lab, we performed PCR test to all the purchased commercial FBS and cell lines we used. The results of PCR indicated that all the commercial FBS were BVDV positive. Further, the cells cultured with these FBS were all BVDV positive (Table 1). Although some FBS and cells were showed weakly positive results, BVDV contamination was determined. The sequencing was proceeded when we obtained the PCR products. The nucleotide blast manifested sequences of those products BVDV-1 or BVDV-3 homologous. While the supporting materials, like PBS and tyrosine, showed BVDV negative. Furthermore, the equine serum from same company was not contaminated. The 5'UTR sequences from these FBS were further analyzed by phylogenetic tree. Phylogenetic analysis showed the 5'UTRs from G-FBS, C2-FBS, J-FBS belonged BVDV-1 (Figure 1). C-FBS and G-FBS were from abroad. Fancifully, the contaminating virus from C1-FBS was

Table 1: The results of RT-PCR of FBS or cells in present study.

Samples	C1-FBS	C2-FBS	G-Equine serum	G-FBS	J-FBS	Tyrisin	DF1	Marc-145	MDBK	BT	M-104	BHK	PBS	M-007
Results of BVDV3 by PCR	+++	+++	-	+	++++	-	+++	+	+	+	+	+	-	+

Notes: C, G, J refer to three commercial serum. C1 and C2 mean different sources. FBS: Fetal bovine serum. DF1, Marc-145, MDBK, BT, M-104, BHK, M-007 are the cell lines cultured using the above serum in the lab.

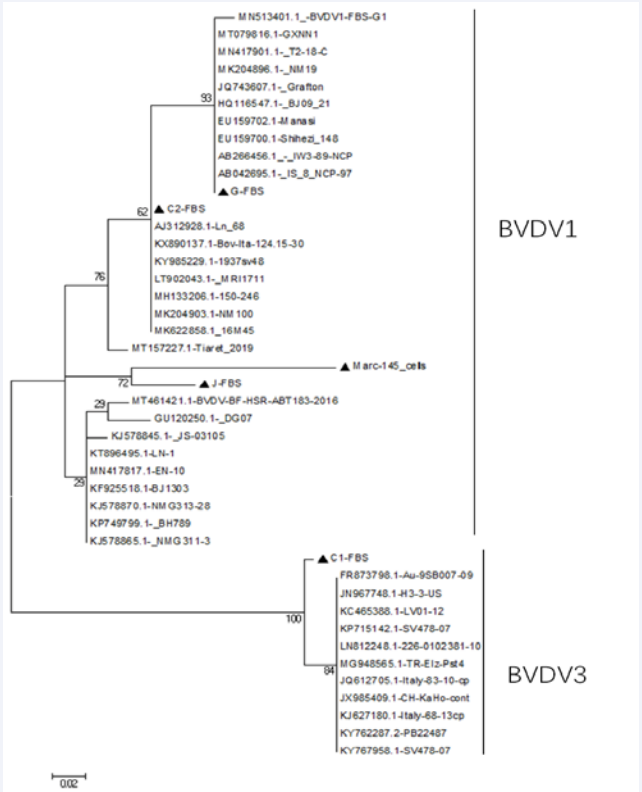

Figure 1 Phylogenetic tree based on the 5'UTR.

Notes: Evolutionary analyses of the 5'UTR from the referenced BVDV-3 strains and isolates from commercial FBS were performed by MEGA7. The phylogenetic tree constructed with 5 isolates sequenced in present study plus 38 reference BVDV strains (shown GenBank No. and name). The studied strains were labeled with a symbol (▲).

typed as BVDV-3-like. We also detected Marc-145 cell line. And the sequence of suspicious fragment was proved similar with the one from J-FBS, which derived from local producer.

The present results indicated the cell culture contaminant was derived from commercial FBS. These BVDV-3-positive FBS batch were firstly reported in China. The detection of atypical pestiviruses in contaminated FBS bring out great concern in cell culture laboratories, with potential effect on BVD control and vaccine biosafety. BVDV-3 has been identified in Brazil [7], Italy [9,10], Bangladesh [11], and Turkey [12]. Detection of BVDV-3 was also reported in FBS originating, processed, and packaged in Australia [13]. Our results demonstrated that the spread of BVDV-3 may transfer with FBS deal. Whether the cattle in China have infected BVDV-3 or not, it needs further investigation. Previous showed the cattle immunized with MLV or killed BVDV1 and BVDV2 vaccines had weak cross immunoreaction to HoBi-like viruses. That is the reason BVDV-3 spread more and more widely. The BVDV-1 or BVDV-2 antibody would likely not suffice to provide protection in BVDV-3 infected cattle [14]. This condition increased the difficulty of clearing BVDV3. Bovine viral diarrhea virus (BVDV) can infect many species, like cows, pigs, sheep, goats [15], and other ruminants, as well as some wild animals. BVDV-1 and BVDV-2 are often detected in these animals. BVDV-3 has rarely been reported in China. In present report, BVDV-3 exist in commercial FBS derived from abroad or local bovine serum. It is a wake-up call for Chinese cattle industry.

The detection and identification of BVDV in the commercial diagnostic reagent or vaccines are critical to the design of monitoring programs for these viruses. The investigation of BVDV-3 and contaminant elimination will benefit for the cattle industry.

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