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NOTE

The prevalent genotypes of bovine viral diarrhea virus in Japan, Germany and the United States of America

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Abstract

Genotypes and subgenotypes of bovine viral diarrhea virus (BVDV) field isolates from Japan, Germany and the United States of America (USA) were identified, and the prevalent pattern of BVDV in individual countries was estimated genetically. Subgenotypes were determined based on phylogenetic analyses of nucleotide sequences of a part of the E2-coding gene of BVDV. Forty-five, 61 and 56 BVDV strains were isolated from naturally infected cattle in Japan, Germany and USA, respectively, between 1980 and 2003. The most prevalent BVDV in these three countries was BVDV-1b. The second most prevalent BVDV strains were 1a, 1d and BVDV-2 in Japan, Germany and USA, respectively. The most prevalent subgenotype 1b in each country constructed individual small clusters in the subgenotype 1b branch in the phylogenetic tree. Although cattle and/or cattle products were moving among the three countries as part of international trade, the distribution of BVDV in the field in each country showed long-standing individual patterns.

Key words : bovine viral diarrhea virus, BVDV, genotype

Bovine viral diarrhea virus (BVDV), a member of the Pestivirus genus of the *Flaviviridae* family, is a major pathogen of cattle and induces various clinical manifestations in cattle. Moreover, the virus is responsible for significant economic losses in the cattle industry around the world[11]. The genome of BVDV consists of a positive stranded RNA of 12.2 to 15.5 kb. Based on the diversity of the viral genome, BVDV has been classified by genotype and subgenotype. Various regions of the viral genome have been utilized for the analysis of genotyping.

Two genotypes, BVDV-1 and BVDV-2, have been identified and are isolated in many countries[1,4,6,9,10,13,21]. The relationship between

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genotype and clinical manifestations is not well known. BVDV-2 seems to be more prevalent in North America than in European and Asian countries[16]. BVDV-1 has been divided into 2 to 11 groups[14,21]. The subgroupings are distinguished based on analyses of the viral genome[12,15]. The development of BVDV infection has been reported throughout the world, however, regional differences in the biological properties of BVDV remain unclear. The clarification of these problems would be useful for the elucidation of the pathogenicity of BVDV and for biosecurity in BVDV eradication program.

In the present study, the gene diversity of the E2-coding region of BVDV was estimated. The antigenicity of BVDV is largely dependent on the E2 antigen. The diversity of E2 gene has also been used to define the genotypes of BVDV isolates[3,12,17]. Therefore, the E2 gene of BVDV field isolates originating from Japan, Germany and the United States of America (USA) were analyzed phylogenetically. Data on the variation of E2 may be useful to clarify the problems mentioned above.

The present study examined 162 BVDV isolates; 45 from the north island of Japan (Hokkaido), 61 from the northern region of Germany and 56 from the eastern region of the USA. The viruses were isolated from naturally infected cattle or bulk tank milk between 1980 and 2003. The details of these viruses have been described previously[15-17].

RNA was extracted from BVDV infected culture cells or supernatant, peripheral blood leukocytes, serum, or somatic cells in bulk tank milk. RNeasy Mini kit (Qiagen, Tokyo, Japan) or QIAamp viral RNA Mini kit (Qiagen) was used for the extraction of RNA according to the manufacturer's instructions. Synthesis of cDNA was carried out using Moloney murine leukemia virus reverse transcriptase (Invitrogen, Tokyo, Japan) and random hexamer primers (Promega, Tokyo, Japan). Specific primers for the E2 gene[17] were used for polymerase chain reaction (PCR). The PCR products were purified and concentrated using Rapid PCR purification systems (Marligen Biosciences Inc., Maryland, USA). The nucleotide sequences of the amplification products were determined with an automated DNA sequencer (ABI PRISM 377 DNA sequencer) using an ABI PRISM BigDye terminator cycle sequencing ready reaction kit (Perkin-Elmer Japan Co., Chiba, Japan) according to the manufacturer's protocol. Each PCR product was sequenced using at least two individual sequencing primers. Previously reported sequencing primers[17] were used in addition to the PCR-primers. As reference sequences for the grouping of genotypes, the corresponding E2 sequences from some additional BVDV were obtained from the GenBank data library (Table 1). The nucleotide sequences were proofread using the computer program GENETYX-MAC (Genetyx co., Tokyo, Japan). The 420 bp fragments of the E2-coding region of each sample were used for phylogenetic analysis. The analysis was

Table 1. Reference strains

Genotype	Strain	Origin	Acc. No.*
1 a	NADL	USA	M31182
1 b	Osloss	Germany	M96687
	CP7	USA	U63479
	Lams738	Germany	AJ302945
1 c	519/93	Germany	AF144610
1 d	Lams735	Germany	AJ302962
	9466/91	Germany	AJ302946
	11158/98	Germany	AJ302995
1 f	4998/89	Germany	AJ302959
1 g	R1891/99	Germany	AJ303001
2 a	890	USA	U18059
2 b	Gi-1/96	Germany	AF104030

* : Accession numbers for the detabase of GenBank

Table 2. Distribution of BVDV genotypes in Japan, Germany and USA

Genotype	Japan	Germany	USA
BVDV 1 a	37.8	1.6	10.7
1 b	46.7	49.2	46.4
1 c	13.3	0	0
1 d	0	39.3	0
others	0	6.6	0
BVDV 2	2.2	3.3	42.9
total	100	100	100

(%)

performed utilizing the PHYLIP package. For visualization of the trees, TREEVIEW version 1.6.0 was used.

The distributions of BVDV subgenotypes in Japan, Germany and the USA are indicated in table 2. BVDV-1b was the most prevalent subgenotype in all three countries. The Japanese field isolates were divided into four subgenotypes. BVDV-1a was second most prevalent subgenotype in Japan. BVDV-1c was identified only among Japanese BVDV isolates. Prototypes of BVDVs such as No. 12, NADL, Oregon C24V and SD-1 were classified as BVDV-1a. These strains have been utilized for the development of vaccine against BVDV. BVDV-1a was not prevalent among field isolates other than those from Japan. More than 180 licensed BVDV vaccines are commercially available in the USA[8]. This might contribute to the low prevalence of the 1a subgenotype in the USA. Although only a few vaccines are available in Japan and Germany, the prevalence of 1a in these two countries differed from each other. Therefore, differences in the distributions of the BVDV subgenotype did not seem to depend on the influence of vaccination. BVDV-1c was identified only in Japan in the present study. However, it was reported that 87 of 89 Australian field isolates were classified in BVDV-1c[9]. Japanese BVDV-1c were isolated from Japanese Black cattle. It was not indicated whether Australian BVDV-1c were isolated from dairy or beef cattle. In other subgenotypes, there is no report concerning breed-specific subgenotype. Because BVDV-1c was initially isolated from deer in New Zealand[2], a species-specific subgenotype might exist, though there is no evidence yet.

German field isolates were classified into many subgenotypes such as 1d, e, f and g, and 1d was the second most prevalent subgenotype in Germany. BVDV-1d was identified only among the German BVDV isolates in the present study. In Denmark and Slovenia, 1d was also the prevalent subgenotype among field isolates[18,19]. In Belgium and Portugal, however, 1b was prevalent[1,4]. Cattle can be easily transported among countries within European Union (EU), and wild animals can move even more easily. These circumstances might contribute to the variety of subgenotypes in Germany.

In the USA, only three genotypes were recognized, and BVDV-2 was the second most prevalent genotype. BVDV-2 was more frequently identified in the USA than in the other two countries. Many BVDV-2 were detected all over the world, and the detection of BVDV-2 was sporadic except in the USA. In the USA, BVDV-2 is a prevalent pathogen for BVDV infection[7,16], and subdivided into two subgenotypes 2a and 2b[3,6]. Typical manifestations of BVDV-2 infection were initially thought to be a hemorrhagic syndrome or peracute severe outbreak. Currently, similar manifestations can be recognized in BVDV-1 infection, and many other clinical signs can be observed in BVDV-2 infection[7,16]. There is, therefore, no relationship between genotype and clinical manifestations.

On phylogenetic analysis, BVDV-1b from each country constituted individual small clusters on the 1b branch of phylogenetic tree

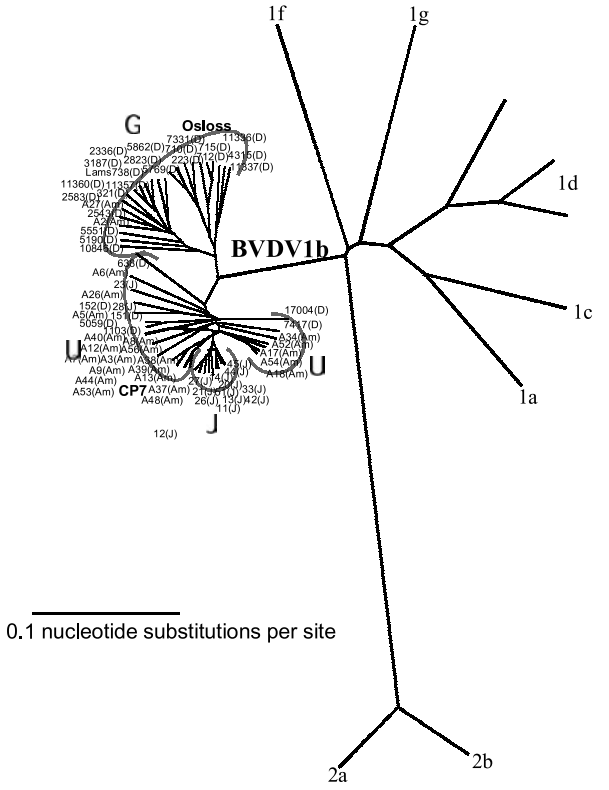

Fig. 1 The phylogenetic tree constructed from 420 nucleotides of the E2-coding region of 77 BVDV-1b field isolates from Japan (21 isolates, indicated as Jp), Germany (30 isolates, indicated as D) and the USA (26 isolates, indicated as Am). Reference strains of each subgenotypes are listed in Table 1.
G: cluster of German isolates, J: cluster of Japanese isolates, U: cluster of American isolates

as shown in Fig. 1. The German 1b group included the classical European BVDV strain Osloss, which was identified in 1965. The American 1b group included the classical American BVDV strain CP7 that was identified in 1988. Japanese 1b was genetically near the American 1b on the phylogenetic tree, although these isolates constructed a different cluster from the American isolates. Moreover, the Japanese cluster was smaller than the cluster of BVDV-1b from the other two countries. This might be only because the number of viruses constructing the 1b branch was greater than those of branches for other subgenotypes[20]. In the present study, part of the E2-coding region was used for subgenotyping. The diversity of the E2 gene reflects the antigenicity of BVDV[5]. Therefore, a distinct cluster of 1b from each country might contribute to different antigenicities of field isolates in each country. The Japanese 1b cluster was smaller than those of the other countries examined in this study. This might suggest that the most prevalent BVDV in Japan had been conserved over a long period. On the other hand, the Japanese 1b cluster looks a subbranch of the American 1b cluster. There was no geographical and chronological relationship among Japanese and American field isolates. This suggested that Japanese and American field isolates were genetically near, although the reasons were not clear.

The genetic diversity of the viral gene is a useful tool for estimating the origin and evolution of field isolates. Before the occurrence of troublesome problem in international trade concerning bovine spongeform encephalopathy, dairy and beef cattle have been moving among the three countries without strict restriction. Semen and embryos are sometimes imported and exported among three countries. To develop an eradication program for BVDV infection, information concerning gene diversity will be important, because it is possible to utilize the gene diversity for the estimation of BVDV invasion. In the present study, it was demonstrated that the distribution of field BVDV from Japan, Germany and the USA showed long-standing individual patterns. This may be useful for the livestock industry to prevent further spread of BVDV during international trade.

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