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NOTE

Bovine viral diarrhea virus infection in a dairy herd with high prevalence of persistently infected calves

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Abstract

A dairy herd including approximately 50 milking cows and 40 heifers and calves was investigated. This herd was detected with high prevalence of calves persistently infected (PI) with bovine viral diarrhea virus (BVDV). Nine PI animals including a milking cow and 8 newborn calves were detected in the herd within 4 months. Prevalence of PI animals in this herd was estimated 7.0% which was very high compared to that estimated in previous reports. All newborn PI calves were strongly suspected to have a single origin of infection as estimated from the homology of the virus genes. The cause of high prevalence could not be clarified. Removal of PI animals and continuous examination of newborn calves were important for the elimination of BVDV from the herd.

Keywords: bovine viral diarrhea virus, BVDV control, persistently infected animals

Bovine viral diarrhea virus (BVDV) is an economically important viral pathogen in cattle that is widespread throughout the world[8)]. BVDV is a single-stranded positive-sense RNA virus belonging to the *Flaviviridae* family, genus *Pestivirus*. Two genotypes, BVDV-1 and BVDV-2, are recognized as distinct species within the genus *Pestivirus*. Based on the genomic sequences of the 5'-untranslated region (5'UTR), N^{pro} and E2 region, several subgenotypes in each genotype are recognized[13,17,21,24)]. The 5'UTR, a highly conserved region of the genome, has shown to be a reliable and reproducible method for genetic characterization of BVDV isolates[17)]. The E2 glycoprotein has been described to be the main target of the neutralizing antibody response

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of the host[3]. It is the least conserved and immunodominant protein[2]. Although the correlation between pathogenicity and subgenotype of BVDV has not been cleared, partial sequencing of the 5'UTR and E2 region was proven to be a useful epidemiological tool for studying BVDV[6,10,19].

BVDV can infect cattle of all ages, with consequences of infection in immunocompetent cattle ranging from subclinical or mild disease to a highly fatal form[1]. Infection of naïve pregnant cows with noncytopathic (ncp) BVDV may result in transplacental infection of the fetus. Adult immunocompetent pregnant cows seroconvert and clear the virus. But the outcome of the infection of the fetus depends on the stage of gestation at which the infection occurred. Infection of the fetus with ncp BVDV after about 150 days of gestation may result in transient infection of the fetus. In contrast, if the developing fetus is infected with ncp BVDV before about 150 days of gestation, the infection may result in the development of immunotolerance to the infecting BVDV strain and persistent infection[1,5,11,14,22]. Persistently infected (PI) animals produce large quantities of virus in their secretions and excretions. These animals are considered to be responsible for most of the spread of BVDV because they are a permanent source of contamination within a herd[7]. The infection is maintained on the herd by PI animals that are immunotolerant against BVDV. The central aspect of a BVDV eradication strategy is to identify PI animals and to eliminate them from the herds. There has been a predominance of studies showing that the prevalence of PI animals ranged from 0.5% to 2.0%[8,9,16]. We detected a dairy herd which produced many PI animals in a short period. In the present study, the status of the high prevalence of PI calves in this herd was described. In addition, the control of BVDV infection was trialed and the practical method for the control was discussed.

The dairy herd surveyed in the present study included approximately 50 milking cows and 40 heifers and calves during the study. All animals in the herd had not been vaccinated against BVDV. There had been no occurrence of BVDV infection in the past few years. All cattle were fed in a tie stall served by 3 workers. There was no known contact with other herds. All cattle in the herd were not grazing about 9 months before PI detection. A newborn PI calf (calf 1) was detected as an index clinical case suffering from nervous manifestations. In order to eliminate BVDV from the herd, all cattle in this herd were examined and 2 PIs including a milking cow and a calf (calf 2) were newly detected. Because of the possibility that non-PI pregnant cattle might carry PI fetuses, surveillance of all calves born in the herd was planned to be performed for 9 months after the last PI detection until birth of all calves subjected to the risk of infection. In the present study, according to the request of the owner of the farm surveillance was extended for 11 months after the last PI detection. The total period of surveillance was over 15 months after the initial examination of all cattle in the herd.

For the identification of PI animal, serum sample was examined to detect the BVDV genes by reverse transcription polymerase chain reaction (RT-PCR) using a primer set from the 5'UTR of the virus gene[15]. Viral RNA was extracted from the serum using QIAamp Viral RNA Mini Kit (Qiagen Inc., Tokyo, Japan) according to the manufacturer's instructions. Synthesis of cDNA was carried out using Moloney murine leukemia virus reverse transcriptase (MMLV-RT, Invitrogen Inc., Tokyo, Japan) and a random hexamer (Promega Inc., Tokyo, Japan). The PI was diagnosed in case of both positive results of RT-PCR in two blood samples taken at approximately 2 weeks interval.

The time course scheme of the PI detection in the herd is shown in Fig. 1. Details about dates of introduction of PI animals to the herd and dates of detection and elimination from the herd are shown in Table 1. During 15 months of surveillance in the period from June 2010 to August 2011, 36 newborn calves were produced in the herd. Six calves were identified as PI (from

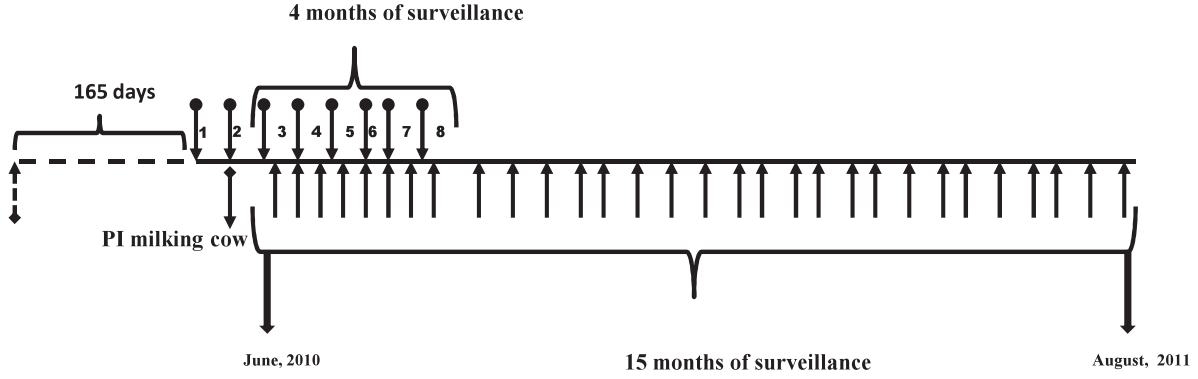

Fig. 1. The time course scheme of the PI detection. Calf 1 was detected at first as an index clinical case. Two more PIs (a milking cow and calf 2) were newly detected. Fourteen calves were born within the first 4 months of surveillance. 6 of them were identified as PI and 8 calves were normal. In the following 11 months of surveillance 22 calves were born and all of them were recognized as not PI. ↕: PI milking cow, ↕: PI calves, ↑: non-PI calves. ⋮: The PI milking cow had been introduced to the herd 165 days before detection (dashed line) of calf 1.

Table 1. Dates of introduction, examination and elimination of PI animals.

PI animal	Introduction to the herd[a]	Examination		Elimination or isolation
		First RT-PCR	Second RT-PCR	
Milking cow	2009–12–05	2010–06–16	2010–06–30	2010–06–23
Calf 1	2010–05–19	2010–05–20	NT[b]	2010–05–20
Calf 2	2010–05–31	2010–06–07	2010–06–21	2010–06–23
Calf 3	2010–06–25	2010–06–30	2010–07–12	2010–07–30
Calf 4	2010–07–13	2010–08–13	2010–08–24	2010–09–24
Calf 5	2010–07–24	2010–08–13	2010–08–24	2010–09–24
Calf 6	2010–08–09	2010–08–13	NT[c]	2010–08–21
Calf 7	2010–08–19	2010–08–24	NT[d]	2010–08–21
Calf 8	2010–09–14	2010–09–21	2010–10–05	2010–09–24

All dates in the table were written in year-month-day format.
[a]The milking cow was purchased from other herd on December, 2009, calves1–8 were born in the herd. Birth dates were indicated except for the milking cow.
[b]Calf 1 died 2 days after birth.
[c]Calf 6 was sold and not examined by second RT-PCR.
[d]Calf 7 was culled from the herd and not examined by second RT-PCR.
PI: persistently infected, RT-PCR: reverse transcription polymerase chain reaction, NT: not tested

calf 3 to calf 8) and 8 calves were identified as not PI within the first 4 months of surveillance. All dams of PI calves were not PI. Three PI calves (calves 1, 6 and 7) could not be examined by a second RT-PCR due to death or elimination from the herd. However, the result of first RT-PCR strongly indicated that these calves were PI as recognized from the intensity of the amplified RT-PCR product reflecting high quantity of virus. Although the examination of calves was done using sera which might contain maternal antibodies to BVDV acquired from colostrum feeding, a strong amplified RT-PCR product was observed in case of PI calves. In the following 11 months of surveillance, 22 calves were born and all of them were recognized as not PI. The PI prevalence represented 7.0% of 128 tested animals in the herd. The surveillance period was extended for 11 months after elimination of the last detected PI calf in the herd according to the request of the owner. If the surveillance period was restricted to 9 months which was the pregnancy period of cattle, the prevalence of PI animals would be higher

than 7.0% as decreasing total number of tested animals. This prevalence was very high compared to that estimated in previous reports (0.5–2.0%)[8,9,16].

The viral genes from PI animals in the herd were identified by RT-PCR using primer sets for the 5'UTR[15] and E2 region[21] of the BVDV genome. The nucleotide sequences of the amplified products were compared. These nucleotide sequences were determined with an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) using an ABI PRISM Big Dye terminator cycle sequencing ready reaction kit (Perkin-Elmer Japan Co., Chiba, Japan). Corresponding sequences obtained from the GenBank data library were referred for the comparison of sequences. Their reference strains were as follow: NADL (accession number M31182: subgenotype 1a), CP7 (U63479: 1b), 519/93 (AF14461: 1c), 721/96 (AF144609: 1d), KS86–1 (AB03375: 1e), 22146/81 (AF144610: 1f), 1891/99 (AJ303001: 1g) and 890 (U18059: 2a). All viruses from PI animals in the herd were classified as BVDV1b which is the most prevalent subgenotype in the world[20]. Also all BVDV genes from PI animals in the present study had no specific genetic characters in phylogenetic analysis (data not shown). 100% nucleotide sequences homology in 220 bp in the 5'UTR was found among BVDV genes from PI animals in the present reported herd. The 5'UTR is a highly conserved region of the genome and it can be used as a tool of molecular epidemiology of BVDV infection[10]. Sequence identity of the BVDV genes based on 5'UTR has been previously reported in other cases[18,23]. Using 288 bp nucleotide segment of the 5'UTR, isolated viruses from the same herd had the same nucleotide sequences or differed only in 1–2 nucleotides[23]. Furthermore, another study compared 237 nucleotide segment of the 5'UTR and several sets of identical sequences were found within the same herd[18]. Although E2 region of BVDV is a highly variable region[3], comparison of 420 bp of the E2 coding region of BVDV genes from PI animals revealed a high degree of sequence homology. The homology was 98.6% to 100% among nucleotide sequences (Fig. 2a) and 96.4–100% among amino acid sequences translated from nucleotide sequences (Fig. 2b). Differences between sequences in the E2 region ranged from 0 to 6 nucleotide substitutions (0–1.4%) and 0 to 5 amino acid substitutions (0–3.6%). Similar results were reported using 188 bp fragment and nucleotide variations estimated 1.6% suggesting that these viruses were having a single common origin[12]. Moreover, it was also reported that nucleotide variations reached up to 1.0% in 389 bases of the E2 region within the same herd[6]. In the present study, therefore, the identity of the BVDV genes from PI animals was strongly indicated.

The PI milking cow was introduced to the herd 165 days before the first PI calf detection. It was reported that infection of susceptible pregnant cows with ncp BVDV before 150 days of gestation, before the development of immune competence, might result in birth of a PI calf[1,5,11,14,22]. The PI milking cow was fed in the herd during the period of risk of immunotolerance against BVDV of all PI calves. All cows in the herd including dams of non-PI calves had the same opportunity to contact with the PI milking cow. There was no known contact with other herds because the cows in the herd had not utilized the public grazing field and not joined cattle exhibitions. There was no occurrence of previous BVDV infection in the herd. The detection of PI calves in the present study was limited to a period of 4 months and was strongly suspected to be caused by a single origin of infection. After introduction of the PI milking cow to the herd, most of cattle could be infected. It was reported that 70% to 100% of susceptible nonvaccinated cattle becoming infected after PI animal exposure[4]. The estimation of antibodies in the present herd could not be preformed because all cattle in the herd were vaccinated after elimination of the PI milking cow before the start of surveillance. It is difficult, therefore, to

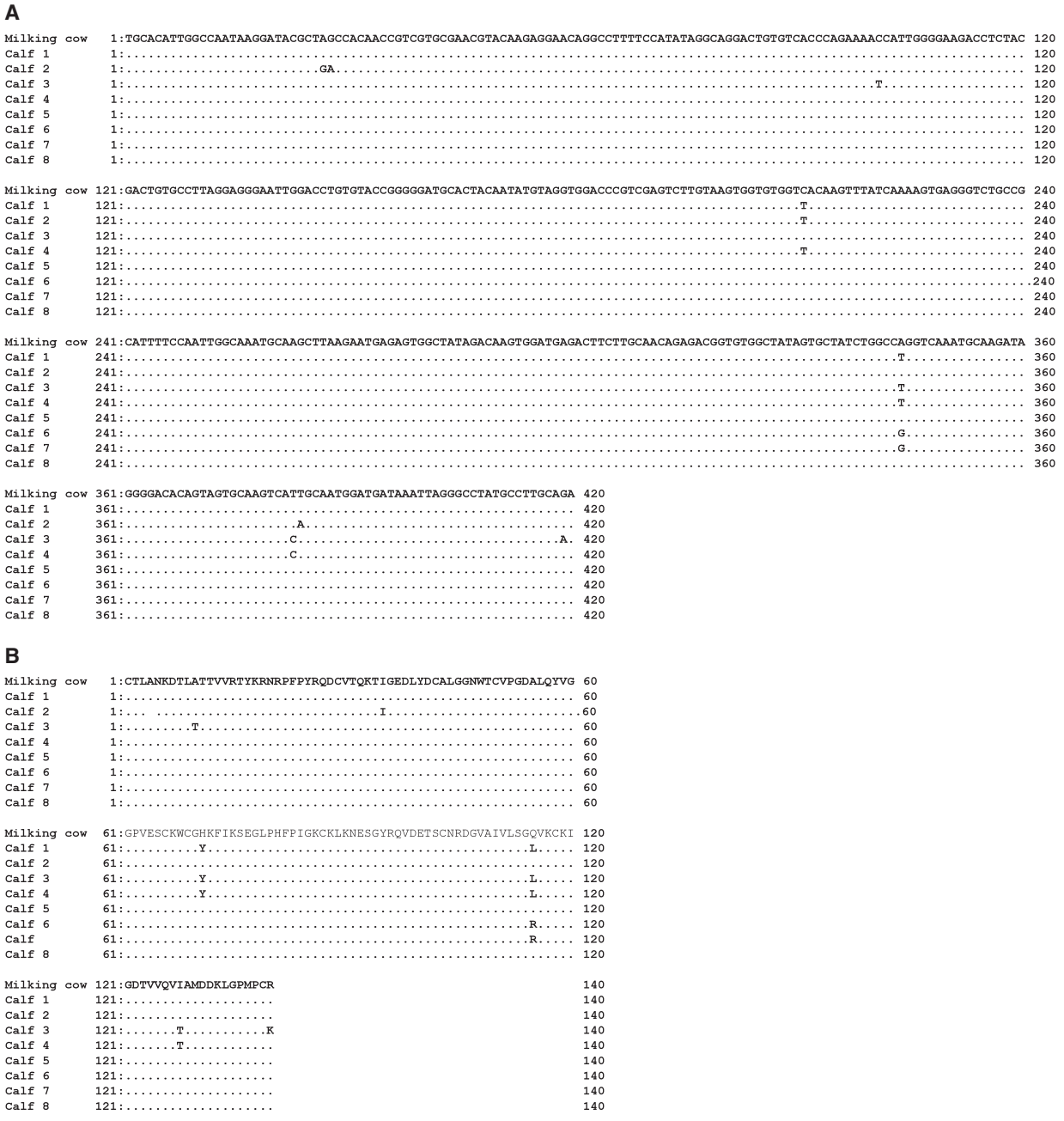

Fig. 2. Comparison of a part of the E2 region of the BVDV genes from PI animals in the herd. (a) 420 bp of nucleotide sequences and (b) 140 amino acid sequences translated from (a).
Dots indicate the identity of the nucleotides.

estimate the spread of BVDV within the herd using antibody titer.

In the present study after elimination of the PI milking cow, 14 calves were born within the first 4 months of surveillance. Six of them were identified as PI and 8 calves were normal. These normal calves might be due to insufficient contact with the PI milking cow or presence of sufficient BVDV specific or cross reacting serum neutralizing antibodies in the dams. In the following 11 months of surveillance, no PI calves were produced inspite of presence of the PI milking cow in contact with some dams of these calves during the period of risk of

immunotolerance. Moreover, some PI calves were kept in the herd for a period more than 2 months after birth until elimination (calves 4 and 5). These calves could be a potential source of BVDV infection. However, these calves were fed individually in isolated hatches and had no direct contact with pregnant cows. Dams producing normal calves inspite of exposure to BVDV infection indicated that they were immune as they might be infected with BVDV before pregnancy and seroconvert. The control method for BVDV in the present reported herd was "test and cull". All PI calves could be detected within a short period. And it was possible to obtain a BVDV-free herd by examination of newborn calves and newly introduced cows to the herd. The preventive effort for BVDV invasion to the herd might be effective to maintain BVDV-free herd status.

In conclusion, the present study described a dairy herd with a high prevalence of PI calves having a single origin of infection in a period of 4 months. The cause of high prevalence could not be clarified. Removal of PI animals from the herd and continuous examination of newborn calves were effective in the elimination of BVDV from the herd.

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