**NOTE** *Virology*

# Bovine Papular Stomatitis Virus (BPSV) Infections in Korean Native Cattle

Jae-Ku OEM[1)], Eun-Yong LEE[1)], Kyoung-Ki LEE[1)], Seong-Hee KIM[1)], Myoung-Heon LEE[1)] and Bang-Hun HYUN[1)]*

[1)]*Animal Disease Diagnostic Division, Animal, Plant and Fisheries Quarantine and Inspection Agency, 480 Anyang-6-Dong, Anyang 430–824, Republic of Korea*

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ABSTRACT. An outbreak of a disease with parapox-like symptoms was reported in South Korea in April 2012. Three of 45 Korean native cattle, age 20–24 months, were affected. Parapoxviruses were detected and identified by electron microscopy and polymerase chain reaction (PCR). To determine the genetic characteristics of the Korean strains, the sequence of the major envelope protein (B2L) was determined and compared with published reference sequences. Phylogenetic analysis revealed that the parapoxvirus strains were closely related to not only isolates from Japan, but also isolates from Germany, Sudan and the United states. This is the first report on an outbreak and the molecular characterization of BPSV in Korea.

KEY WORDS: *B2L* gene, bovine popular stomatitis virus, cattle, phylogenetic analysis.

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*Parapoxvirus*(PPV) belongs to the family *Poxviridae*, which are oval, enveloped, double-stranded DNA viruses. The genus also includes bovine papular stomatitis virus (BPSV) and pseudocowpox virus (PCPV) of cattle, orf virus (ORFV) of sheep and goats, parapoxvirus of red deer in New Zealand (PVNZ) and squirrel parapoxvirus (SPPV) [3]. Bovine papular stomatitis (BPS) is a common epitheliotropic viral disease of calves characterized by the formation of papules or nodules that progress to vesicles and then crusts or scabs on the lips, gingiva and tongue. It was reported that the teats are affected and mouth lesions aren't observed in some case [11]. Additionally, severe esophagitis and dermatitis in cattle were reported recently [7, 8]. Zoonotic PPV infection characterized by nodules and pustules mainly on the hands, face and arms has been reported [1, 4, 11].

To diagnose PPV, clinical signs, virus isolation and electron microscopy are commonly used, as well as serological tests. Recently, polymerase chain reaction (PCR) and DNA sequencing have been used to detect and analyze PPVs. The major envelope protein *B2L* gene has been used to reveal genetic variation and to characterize PPV [2, 11].

Orf virus infection was previously reported in dairy goats in Korea [10].

Parapoxvirus infections in domestic and wild ruminants have been reported in China and Japan [5, 7, 9, 14, 15]. However, no parapoxvirus infection has been reported in cattle in Korea.

In this study, we report an outbreak of BPSV infection in cattle and verify the identity of the virus using molecular diagnostic methods. This is the first report of the outbreak and the molecular characterization of PPV in cattle in Korea.

In April 2012, three of 45 Korean native cattle at a farm in Gyeongbuk province presented with mildly erosive papules, coalescent scabby erosions and ulcers mainly on the muzzle (Fig. 1a). The affected farm held cattle for beef production and breeding. The mean temperature at the time of the outbreak was approximately 18°C, and weather was dry and clean. The disease morbidity was 6.67% (3/45), and the affected cattle were 20–24 months old. All affected cattle recovered 13–18 days after the clinical signs first appeared without treatment. Clinical samples of the noses and lips of the affected cattle were sent to the laboratory and stored in a −70°C freezer until the samples were used for a further study.

The tissue samples were homogenized mechanically in phosphate-buffered saline (PBS) in a tube using a pellet pestle device. The homogenates were then centrifuged at 3,000 × *g* for 5 min, and the supernatant was collected and stained with 2% phosphotungstic acid to examine the virus using transmission electron microscopy.

PCR using the primers PPP-1 (5'-GTCGTCCACGATGAGCAGCT-3') and PPP-4 (5'-TACGTGGGAAGCGCCTCGCT-3') was also performed, as previously described [6]. The total viral DNA was extracted from the clinical samples using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions.

The amplified DNA fragment was purified using an Agarose Gel DNA Extraction Kit (INtRON, Daejeon, Korea) and subcloned into the vector pGEM-T (Promega, Madison, WI, U.S.A.) according to the manufacturer's instructions. Automated nucleotide sequencing of the *B2L* gene inserted into the vector was performed on an ABI 3130XL genetic analyzer (Applied Biosystems, Foster City, CA, U.S.A.) with a Big Dye Terminator cycle sequencing kit (Applied Biosystems). All nucleotide positions were confirmed based on three or more independent sequencing reactions in both directions.

*CORRESPONDENCE TO: Hyun, B.-H., Viral Disease Diagnostic Laboratory, Animal Disease Diagnostic Division, Animal, Plant and Fisheries Quarantine and Inspection Agency, 480 Anyang-6-Dong, Anyang 430–824, Korea.
e-mail: hyunbh@ Korea.kr

The partial *B2L* gene sequences of the Korean BPVS strains were aligned with those of parapoxvirus sequences obtained from GenBank using BioEdit software (Ibis Biosciences, Carlsbad, CA, U.S.A.). A phylogenetic analysis was conducted using BioEdit and Molecular Evolutionary Genetics Analysis (MEGA) 4.0, with bootstrap values calculated from 1,000 replicates [13]. The phylogenetic algorithm used for building the tree was the neighbor-joining method.

Parapoxvirus was detected using a PCR method, and the amplified 590-bp PCR products were visualized by electrophoresis (data not shown). The virus particles with the characteristic size (approximately 200 × 150 nm) and an oval-shaped morphology of parapoxviruses were observed using transmission electron microscopy (Fig. 1b).

The sequenced PCR products shared higher percent identity with BPSV than with PCPV, PVNZ or ORFV. The sequence comparison of the three Korean BPSV strains demonstrated a high degree of identity (99.8%) between each strain and other BPSV strains. The deduced amino acid sequences of the partial *B2L* gene of the Korean BPSV strains and the other strains were aligned using BioEdit. Unique amino acid substitutions were found at three positions: $V^{3}$→G, $G^{5}$→R and $E^{14}$→S.

In the phylogenetic analysis based on the partial *B2L* gene, parapoxviruses were divided into two different groups. Korean BPSV strains were clustered together with several BPSV isolates. The Korean strains were phylogenetically closer to the isolates from Japan (Fig. 2). The Korean BPSV strains and Japanese Ishikawa-S strain (AB044801) showed 97.2–97.4% and 94.6–95.1% similarity at the nucleotide and amino acid levels, respectively. By contrast, the orf virus (ORFV/2009/Korea) isolated from a goat in 2009 clustered in different group, despite having the same geographic origin.

Parapoxvirus infections are usually considered to be diseases that should be differentiated from important vesicular diseases in ruminants. Bovine papular stomatitis does not have to be reported to the World Organization for Animal Health (OIE), and infections in humans have likely been underestimated. However, several papers have reported the presence and wide distribution of parapoxvirus infection in cattle and humans [1, 2, 7, 8, 11]. Recently, severe, atypical cases of parapoxvirus infection were reported [7, 8]. Therefore, the importance of parapoxvirus infection in ruminants and human should be re-evaluated.

No parapoxvirus infection in cattle and human has been reported in Korea, and no vaccination has been implemented to control these diseases. However, it is possible that more parapoxvirus infection cases would be found in cattle and humans, if an extensive virological survey was performed in Korea. Farmers and others working with livestock, especially ruminants, should be aware of the possibility of PPV infection.

Clinical sings, virus isolation and electron microscopy are used to identify parapoxviruses. However, the classification of parapoxviruses according to host and clinical signs does not always reflect the classification obtained with molecular analysis [8]. Recently, PCR methods have been used to detect the viruses. In this study, BPSV was confirmed by genomic analysis, including sequencing and sequence alignment. Using these methods, ORFV infection was reported in dairy goats in Korea [10]. Molecular methods, such as PCR

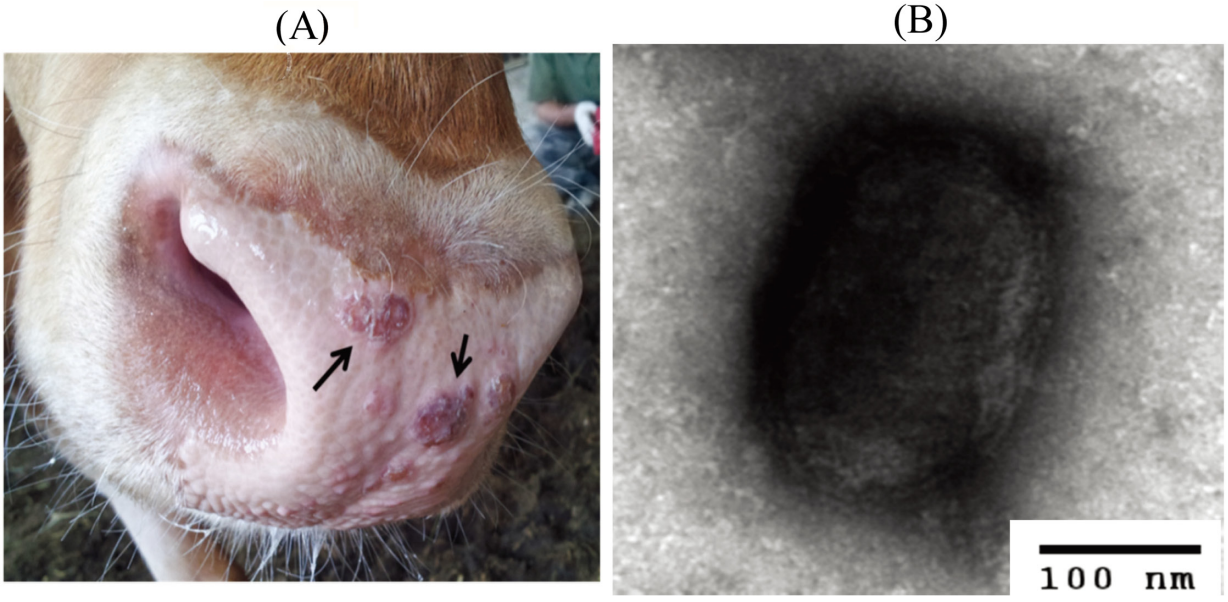


Fig. 1. (A) Typical clinical cases of BPSV infection in Korean native cattle. (B) An electron micrograph of a BPSV particle showing the typical oval-shaped morphology (Bar=100 nm).

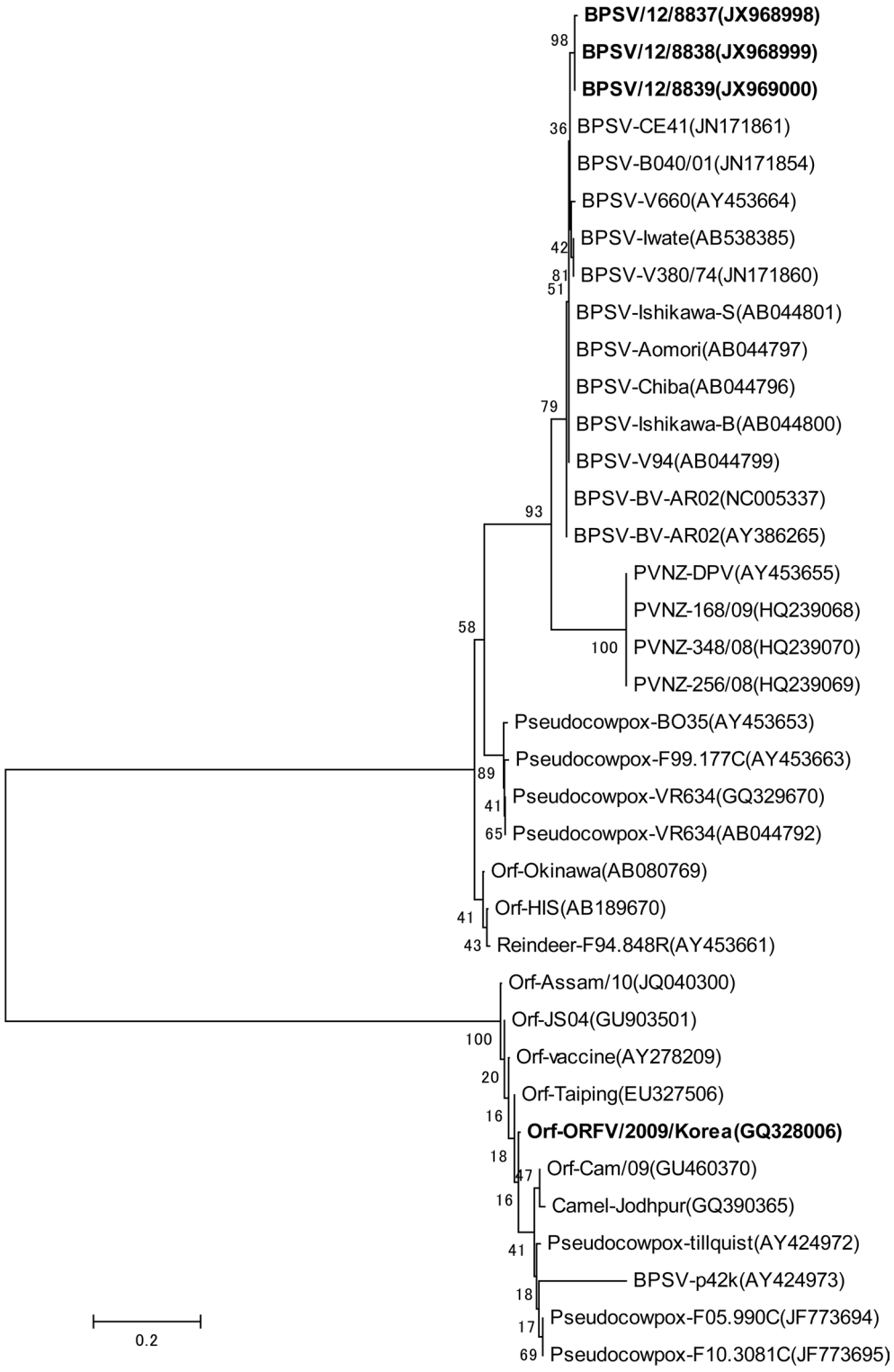


Fig. 2. Phylogenetic analysis of different parapoxviruses based on amino acid sequence of the partial *B2L* gene. The amino acid sequences of diverse BPSV were aligned using BioEdit and MEGA 4. The consensus phylogenetic tree was obtained using amino acid sequence distance and the neighbor-joining method. One thousand bootstrap replicates were performed, and the bootstrap values were displayed above the tree branches; only bootstrap values>70% are shown.

and genomic analysis, are suitable for detecting pathogens that were reported previously.

It has been proposed that BPSV and PCPV infections be referred to as "PPV infection", because it is impossible to determine the causative PPV until genetic studies are performed [7]. In this study, the PPVs were generally divided into individual groups (ORFVs, PVNZ, BPSVs and PCPVs). Some of ORFVs, BPSVs and PCPVs belonged to both groups. This suggests that the proposal to use "PPV infection" is correct. However, further studies should examine whether the use of the partial *B2L* gene for molecular analysis is suitable.

In Japan, the morbidity of parapoxvirus infection is very high in sheep and cattle in serological survey [10, 12]. Recently, severe dermatitis on cattle teats caused by BPSV was reported in Japan [8]. Also, zoonotic transmission of bovine papular stomatitis virus was reported from calf to human in United Kingdom (UK) [5]. Cases clinically compatible with BPSV infection have been frequently reported by veterinarian, yet many cases have not been confirmed in laboratory in Brazil [3]. It has been known that BPSV infection is common in young cattle. However, several cases of BPSV have recently been reported in adult cattle [3, 8]. BPSV infections occurred in Korean cattle approximately 2 years old. It is not clear why these BPSV infections occurred in adult cattle.

Although BPSV infection in cattle is distributed worldwide, the economical importance of the disease has been rarely investigated. In Korea, BPSV infection has not been considered as important disease economically as well. However, because BPS is zoonotic disease, the continuous monitoring is required to decrease the economic loss in cattle industry and human health.

Although this study examined only one case from Korean native cattle showing clinical signs, the three Korean BPSV strains were closely related to isolates from Japan, Sudan and the United States in the phylogenetic analysis based on the *B2L* gene. However, it is difficult to determine the precise route by which the Korean BPSV strains were introduced into Korea based only on molecular analysis. At present, the import of all ruminants from Japan and United States into Korea has been prohibited, because of an outbreak of bovine spongiform encephalopathy (BSE) in Japan and United States. Live cattle have been imported from Australia and New Zealand. Therefore, more epidemiological data on the distribution of BPSV in Korea and neighboring countries are needed. This is the first report of an outbreak and the molecular characterization of BPSV in Korea.

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