

## 学 位 論 文 要 旨

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[論文題名]			
<b>Studies on the Diversity of Papillomavirus in <i>Bovinae</i></b> ウシ属におけるパピローマウイルスの多様性に関する研究			
① Characterization of novel bovine papillomavirus type 12 (BPV-12) causing epithelial papilloma. Archives of Virology, 157(1):85-91,2012 ② <i>Bos grunniens</i> papillomavirus type 1: a novel deltapapillomavirus associated with fibropapilloma in yak. Journal of General virology, 94:159-165,2013 ③ Bovine papillomavirus type 10 with a deletion associated with a lingual papilloma in a cow. The Veterinary Journal, accepted			
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<p>Papillomaviruses (PVs) are widespread in nature and can infect most mammalian species as well as birds and turtles. Since the first PV was identified in cottontail rabbits in the 1930s, to date, about 200 PV types have been identified. The biological subfamily <i>Bovinae</i> includes a diverse group of 10 genera of medium to large-sized ungulates, including domestic cattle, bison, African buffalo, the water buffalo, the yak, and the four-horned and spiral-horned antelopes. In the animal kingdom, papillomas occur more commonly in domestic species of <i>Bovinae</i>, such as cattle and yak, than in any other domestic animal. Current PV studies suggest that there are still many unknown aspects of PV infection in <i>Bovinae</i>, and many more PV types are waiting to be discovered. Accurate information on PV type and its association with disease will provide further understanding of the diversity, biology and pathogenicity of the family <i>Papillomaviridae</i>. In the present study, we focused on PV infection in <i>Bovinae</i>, and successfully identified two novel PV types: bovine papillomavirus type 12 (BPV-12) and <i>Bos grunniens</i> papillomavirus type 1 (BgPV-1). Meanwhile, bovine papillomavirus type 10 (BPV-10) was initially demonstrated in mucosal papilloma, whereas previously this type has only been associated with cutaneous papillomas in cattle.</p> <p>For BPV, 11 types (BPV-1 to -11) have been characterized so far and assigned to four genera: members of the genus <i>Deltapapillomavirus</i> (BPV-1 and -2) cause fibropapillomas in cattle and sarcoids in horses, and they exhibit a somewhat broader host range and tissue tropism than other types. Members of the genus <i>Xipapillomavirus</i> (BPV-3, -4, -6, -9, -10 and -11) are restricted to cattle and infect only epithelial cells to induce true epithelial papillomas. Members of the genus <i>Epsilonpapillomavirus</i> (BPV-5 and -8) appear to cause both fibropapillomas and true epithelial</p>			

papillomas, and a virus not assigned to a genus (BPV-7) has been detected in a cutaneous papilloma. In addition, at least 16 novel putative BPV types (BAA1 to -4, BAPV3 to -5, BAPV7 to -10, BAPV11MY and BPV/BRUEL2 to -5) have been detected in healthy skin swabs or cutaneous warts from cattle kept in Sweden, Japan and Brazil.

BAA1 is one of the putative BPV types and was first detected in a healthy skin swab. So far, there has not been a report regarding the pathogenicity of BAA1, and very little information is available. In the present study, one papilloma sample was collected from the tongue of one infected cow. Histopathological and immunohistochemical analyses showed that it is an epithelial papilloma and contains PV antigen. Then PCR was performed with primer pair FAP59/FAP64 and the PCR product was sequenced. Sequence similarity analysis using the BLAST tool (NCBI) revealed that the subgenomic fragment was related to putative BPV type BAA1. Subsequently, the whole genome of this virus strain was amplified and sequenced. The viral genome is 7197 base pairs in length and contains five early ORFs (E1, E2, E4, E7 and E8), three late ORFs (L1, L2 and L3), and a long control region that possesses replication regulatory elements. Meanwhile, mRNA of each gene was detected in the papilloma sample. Based on the ORF of the L1 gene, phylogenetic analysis showed that BAA1 is a novel BPV type and should be classified as a member of the genus *Xipapillomavirus*. In 1995, the definition of novel PV types was released from the International Papillomavirus Workshop held in Quebec. A PV strain can be recognized as a novel type if the complete genome has been cloned and the DNA sequence of the L1 ORF shares less than 90% similarity with the closest known PV type. Based on this criterion, BAA1 was designated as bovine PV type 12 (BPV-12).

The yak, *Bos grunniens*, is herbivorous and predominantly inhabits the Qinghai–Tibetan Plateau, known colloquially as the ‘roof of the world’. Yaks are regarded as one of the world’s most remarkable domestic animals, as they thrive in extremely harsh, deprived conditions while providing a livelihood for local people. Although PVs have been widely reported in vertebrates, they have not yet been reported in yaks. We report, for the first time, a novel *deltapapillomavirus* that was associated with fibropapilloma in yak herds on the Qinghai–Tibetan Plateau. Six skin papilloma samples were collected and examined using histopathology, immunohistochemistry and PCR assays. The samples were identified as fibropapilloma and were found to contain PV antigens. Full-length PV genome sequences were amplified and sequenced from samples Qh-1 to -6. Each of the samples contained a unique complete PV genome sequence, which was confirmed by rolling-circle amplification (RCA) and long PCR. Full-length genomes of all six strains were 7946 bp, with a G+C content of 44.4 %. Sequence alignment revealed 99.9–100% nucleotide sequence identity among them, and there was no specific variation that caused significant differences in ORFs, motifs, etc. They possessed a typical PV genome organization, consisting of the long control region (LCR) and

early and late regions. Whole-genome sequence alignments revealed that the closest related PVs were BPV-1 (82.3% similarity) and BPV-2 (82.1%). Based on the criterion of PV denomination which was described above, the PV strain identified in this study should be designated a novel PV type. Because it was isolated from *Bos grunniens*, the virus was designated *Bos grunniens* PV type 1 (BgPV-1). It is classified in the Delta-4 species of the genus *Deltapapillomavirus* based on phylogenetic analysis of the L1 ORF.

Members of the papillomavirus family are tissue-specific, and different types of papillomavirus infections usually cause papillomas in different parts of the body. To date, BPV-10 has only been associated with cutaneous lesions in cattle. In present study, one papilloma sample MY-P55 was collected in a slaughterhouse from the tongue of an infected cow. Histopathological and immunohistochemical analyses was carried out. Based on the histopathological characteristics and the presence of BPV antigens in the neoplastic cells, the tumour was diagnosed as epithelial papilloma due to BPV infection. Meanwhile, DNA was extracted and detected by PCR. The sample was found to be positive for primer set FAP59/FAP64. After sequencing the PCR product, sequence similarity analysis using the BLAST tool (NCBI) revealed that the subgenomic fragment was related to BPV-10 and shared 99.5% nucleotide identity with the previously reported strain (AB331651). Moreover, the rolling circle amplification (RCA) product of the DNA of MY-P55 was used as a PCR template with type-specific primers and the consensus primer set FAP59/FAP64 to confirm that no other types of BPV were present in this lesion. This study is the first to report that BPV-10 is associated with mucosal papilloma. This finding suggests that BPV-10 does not have strict tissue-specificity and that it may be associated not only with cutaneous papilloma but also with mucosal papilloma. Whole genome analysis showed that the genome of this BPV-10 strain has a 129-base-pair deletion in the E1 open reading frame, which was confirmed by Southern blot, PCR and reverse transcription-PCR. The result illuminates a number of the properties of BPV-10 and provides further information for PV studies.

In conclusion, the present study identified two novel PV types: BPV-12 and BgPV-1, which were associated with mucosal papilloma in the tongue of cattle and cutaneous papilloma in yak, respectively. Meanwhile, BPV-10 was initially demonstrated in mucosal papilloma and a number of properties were illuminated. This study not only contributes to the PV diversity research in *Bovinae* but also provides further understanding of the diversity, biology and pathogenicity of the family *Papillomaviridae*.

(1,226 words)

備考 論文要旨は、和文にあつては2,000字程度、英文にあつては1,200語程度とする。