Six bovine papillomavirus (BPV) types and 16 putative BPV types have been reported previously. Here, the complete genome sequence of BAPV6, a novel putative BPV type isolated from cattle in Japan, was determined by using multiple-primed rolling-circle amplification. The genome consisted of 7412 bp (G+C content of 46 mol%) that encoded five early (E1, E2, E4, E6 and E7) and two late (L1 and L2) genes, but did not encode the E5 gene. The E6 protein contained a non-consensus CxxC(x)32CxxC and a consensus CxxC(x)29CxxC zinc-binding domain, and the E7 protein lacked the LxCxE motif. The nucleotide sequence of the L1 open reading frame (ORF) was related most closely (57–58 %) to the L1 ORF of member(s) of the genera Betapapillomavirus, Gammapapillomavirus and Pipipapillomavirus. Phylogenetic analysis based on the complete L1 ORF suggests that BAPV6 should be classified in a novel genus in the family Papillomaviridae as BPV-7.

BPV-1 and -2 contain ORFs E6 and E7 in the early region of the genome, whereas ORF E5 is localized between the early and late genes (ERL). These ORFs encode proteins implicated in the transformation of host cells (Schiller et al., 1986). However, BPV-3, -4 and -6 lack the E6 and E5 ORFs (generally found in the ERL), but have the E8 or E5 (formerly E8) ORF in place of the E6 ORF (Jackson et al., 1996; Morgan & Campo, 2000). BPV-5 contains the E6 and E7 ORFs and a putative E5 ORF in the ERL. The E6 proteins of all BPV types contain two zinc-binding or putative zinc-binding domains that seem to be essential for the formation of multimerized complexes. The E7 proteins of most PVs, including BPV-3, -4 and -6, contain the LxCxE motif implicated in the immortalization and transformation of the host cell (Chan et al., 2001; Dahiya et al., 2000; Dick & Dyson, 2002). However, the E7 proteins of BPV-1, -2 and -5 lack this motif (Narechania et al., 2004).

Recently, the multiple-primed rolling-circle amplification (RCA) method has been optimized for rapid amplification of circular DNA (Dean et al., 2001) and used for PV DNA amplification (Rector et al., 2004a, b, 2005). In this study, the complete genome of BAPV6, a putative novel BPV type, was determined by using PCR and RCA methods. Data from sequencing and phylogenetic analysis suggest that BAPV6 is a novel BPV type that should be classified in a novel genus of the Papillomaviridae; BAPV6 was thus designated BPV-7.

BPV-7 was isolated from a cutaneous papilloma found in cattle and detected in two of the 15 (13 %) papilloma specimens and in eight of the 24 (33 %) healthy teat skin swab samples, suggesting that BPV-7 is the most prevalent PV type found in cattle in Japan. BPV-7 DNA was extracted from the biopsy sample of a teat that did not harbour any other BPV or putative BPV types (Ogawa et al., 2004).
DNA was amplified by PCR using primer pairs FAP59/ MY09 (Forslund et al., 1999; Manos et al., 1989) and by RCA using a TempliPhi 100 amplification kit (Amersham Biosciences). ORF analysis was performed using the ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and similarity searches were performed with the NCBI BLAST server (version 2.28) and GenBank. The complete genome of BPV-7 consisted of 7412 bp, and the genome contained E6, E7, E1, E2, E4, L1 and L2 ORFs (Fig. 1a). The length of the ELR was 205 bp. However, the genome lacked the E5 ORF, which is known to encode a small transforming protein.

Sequence analysis revealed canonical polyadenylation signals (AATAAA) located at nt 4077–4082 and 7110–7115 for the early and late mRNA, respectively (Fig. 1a). In the upstream regulatory region (URR), three E2-binding sites with the consensus sequence [ACC(N)₆GGT], which were found in the URRs of BPVs in the genus Xipapillomavirus, were located at nt 45–56, 7129–7140 and 7215–7226. A binding site for nuclear factor 1 (TTGGCA) (Wingender, 1988) was also present at nt 7226–7231. A TATA box-like sequence (TATATTA) was found at nt 57–63 (Fig. 1b).

![Fig. 1. (a) Schematic representation of BPV-7 genome organization. Each ORF is represented as a rectangle. Numbers represent the nucleotide positions of the start and stop codons of BPV-7. Arrowheads indicate the locations of polyadenylation signals for the early and late mRNAs. (b) DNA motifs found in the URR. Numbers represent nucleotide positions in the URR of the BPV-7 genome. The rectangle indicates the polyadenylation signal (AATAAA) for the late mRNA. Shaded boxes indicate the E2-binding motif [ACC(N)₆GGT]. A single underline indicates the nuclear factor-binding site (TTGGCA). A double underline indicates the TATA box-like sequence (TATATTA).](image)

**Table 1.** DNA sequence similarity (%) of the L1 ORF and full-length genome of BPV-7 to those of some PVs that are related closely to BPV-7

<table>
<thead>
<tr>
<th>Genus</th>
<th>Papillomavirus</th>
<th>L1 ORF (%)</th>
<th>Full-length genome (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betapapillomavirus</td>
<td>All types</td>
<td>57–58</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>HPV-15</td>
<td>40</td>
<td>ND</td>
</tr>
<tr>
<td>Gammapapillomavirus</td>
<td>All types except HPV-4</td>
<td>52–53</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>HPV-4</td>
<td>57</td>
<td>41</td>
</tr>
<tr>
<td>Pipapillomavirus</td>
<td>HaOPV</td>
<td>57</td>
<td>41</td>
</tr>
<tr>
<td>Xipapillomavirus</td>
<td>BPV-3</td>
<td>53</td>
<td>42</td>
</tr>
</tbody>
</table>

ND, Not determined.
Recently, numerous PV types representing novel, as-yet-unnamed PV genera have been published in GenBank: canine CPV2 and CPV3 (Tobler et al., 2006), goat ChPV-1 (van Doorslaer et al., 2006), multimammate mouse MCPV2, harvest mouse MmPV, Egyptian fruit bat RaPV-1 and bottlenose dolphin TtPV2 (Rehtanz et al., 2006).

Pairwise DNA sequence alignments were calculated by using the GAP program of Alignment App (http://genome.cs.mtu.edu/align/align.html). The results showed that closely related PV types, i.e. those that shared 57–58% similarity with the BPV-7 L1 ORF, were all of the human PV (HPV) types in the genus Betapapillomavirus, HPV-4 in the genus...
**Gammapapillomavirus** and hamster oral papillomavirus (HaOPV) in the genus *Pipipipomavirus*; other members of the genus *Gammapapillomavirus* had similarities of 52–53% (Table 1). The L1 ORFs of novel PV types ChPV-1 and MmPV showed similarities of 54–56% with the BPV-7 L1 ORF. Similarities between the full-length sequence of BPV-7 and those of HPV-15, HPV-4, HaOPV and BPV-3 were 40, 41, 41 and 42%, respectively. A phylogenetic tree of L1 ORF sequences was constructed using MEGA version 3.1 based on the neighbour-joining method (http://www.megasoftware.net/mega.html) (Kumar et al., 2004). The BPV-7 L1 ORF was related distantly to other L1 ORFs in the phylogenetic tree, which was constructed with 54 PV L1 ORFs, including the L1 ORFs of seven novel PV types (Fig. 2). These results suggest that BPV-7 represents a novel genus in the family *Papillomaviridae*.

The BPV-7 E6 ORF encoded a protein consisting of 142 aa, which contained a non-consensus CxxC(x)33CxxC zinc-binding domain and a consensus CxxC(x)29CxxC zinc-binding domain separated by 38 aa. The E7 ORF encoded a 104 aa protein that lacked the LxCxE motif, but contained a consensus zinc-binding domain, CxxC(x)29CxxC. Lack of the LxCxE motif was also found in E7 ORFs of HaOPV, HPV-4, -50 and -60, and all other members of the genus *Gammapapillomavirus*. The other non-consensus zinc-binding motifs or domains, CxxxC(x)29CxxC, CxC(x)29 CxxC and CxxC(x)30CxxC, were also found in some of these PVs.

It has been reported that the ELR of BPV-1 and other ungulate PVs contain an E5 ORF (de Villiers et al., 2004). The E5 ORF of BPV-1 (Schiller et al., 1986), the E9 ORFs of other transforming unguate PVs (Eriksson et al., 1994) in the genus *Deltapapillomavirus* and the E5 ORFs of some HPVs, including HPV-6 and -16 in the genus *Alphapapillomavirus*, encode a transforming protein containing transmembrane domain(s) (Straight et al., 1993; Conrad et al., 1993). BPV-3, -4 and -6 lack the E6 ORF and contain an E8 or E5 ORF (formerly E8 ORF) in the position of the E6 ORF. The BPV-4 E5 ORF consists of 42 aa, induces anchorage-independent growth of infected cells and suppresses contact inhibition (O’Brien et al., 1999; Morgan & Campo, 2000).

In the absence of the E5 ORF, fibroblast transformation may be mediated by cooperation between the E6 and E7 ORFs (Neary & Dimiao, 1989). The E6 ORFs of most PVs contain two CxxC(x)29CxxC domains, separated by 35–37 aa. These domains bind zinc through cysteine residues and can act as dimerization/multimerization domains of E6 proteins (Barbosa et al., 1989; Grossman & Laimins, 1989). The CxxC(x)29CxxC domain found in most of the E7 proteins can also act as a dimerization/multimerization domain (Barbosa et al., 1989; Clemens et al., 1995; McIntyre et al., 1993). BPV-7 lacks the E5 ORF. Thus, it may be assumed that the non-consensus-structured domain CxxC(x)33CxxC of the BPV-7 E6 protein, as well as CxxC(x)29CxxC in the BPV-5 E7 protein and CxxxC(x)29CxxC in the HPV-45 E7 protein, could function as zinc-binding domains.

The LxCxE motif found in the E7 protein is a canonical pRb-binding motif and has been implicated in the immortalization and transformation of the host cell (Chan et al., 2001; Dahiya et al., 2000; Dick & Dyson, 2002). Most HPV E7 proteins use a homologous LxCxE motif to bind to the pocket region of pRb, p107 and p130 and prevent interactions with the transcription factor E2F-1 (Helt & Galloway, 2003). However, the E7 proteins of artiodactyla PVs, including BPV-1, -2, -5, European elk PV (EEPV), deer PV (DPV) and reindeer PV (RPV) (Narechania et al., 2004), and BPV-7 lack this motif.

In addition to the PVs isolated from papilloma specimens, large numbers of putative HPV and animal PV types have been detected by PCR from the healthy skin of humans and other animals (Antonsson & Hansson, 2002; Antonsson et al., 2003; Astori et al., 1998; Ogawa et al., 2004). This shows a latent or subclinical infection of skin with PV and their commensal nature. BPV-1, -3, -5 and -6, as well as BPV-7, have been detected in swab samples of healthy teat skin without apparent papilloma, indicating latent or subclinical infections, in addition to papilloma-inducing infections, of these PV types.

The BPV-7 L1 ORF shows high nucleotide sequence similarity to the L1 ORFs of HPVs of the genera *Betal-papillomavirus* and *Gammapapillomavirus* and HaOPV of the genus *Pipipipomavirus*, but appears to have a distant relationship to other PVs in the phylogenetic tree, suggesting that BPV-7 should be classified in a novel genus of the family *Papillomaviridae*.

### References


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