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Short Communication

Bovine papillomavirus type 10 with a deletion associated with a lingual papilloma in a cow

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ABSTRACT

Different types of papillomavirus usually cause papillomas in specific tissues. Previously, bovine papillomavirus (BPV) type 10 has been associated specifically with cutaneous papillomas in cattle. In this study, BPV-10 was detected in a papilloma on the tongue of a cow. Whole genome analysis demonstrated that the sequence of this BPV-10 strain had a 129 base pair deletion in the E1 open reading frame, which was confirmed by Southern blot analysis, PCR and reverse transcriptase-PCR.

Most papillomaviruses (PVs) are highly species- and tissue-specific, but some can cause cross-species infection, e.g. bovine papillomavirus (BPV) types 1, 2 and 8 (Freitas et al., 2011). To date, BPV-10 has only been associated with cutaneous lesions (Hatama et al., 2008; Rai et al., 2011; Batista et al., 2013). Here we report infection with BPV-10 in a papilloma on the tongue of a cow and demonstrate the presence of a deletion in the early region E1 open reading frame (ORF) of the virus genome.

A papilloma (MY-P55) was collected at an abattoir from the tongue of a 32-month-old Japanese black cow in Miyazaki Prefecture, Japan. DNA was extracted with the DNeasy Blood & Tissue Kit (Qiagen) and the sample was positive for BPV by PCR using the oligonucleotide primer pair FAP59/FAP64 (Forslund et al., 1999), but negative using primer pair MY09/MY11 (Manos et al., 1989). The 428 base pair (bp) FAP59/FAP64 PCR product was cloned and sequenced; sequence analysis using BLAST[1] demonstrated 99.5% nucleotide identity with BPV-10 (AB331651) (Hatama et al., 2008). The rolling circle amplification (RCA) product of the DNA of MY-P55 was used as a PCR template with type-specific primers (Carvalho et al., 2012) and the consensus primer set FAP59/FAP64 to confirm that no other types of BPV were present in this lesion.

Sections of the papilloma were stained with haematoxylin and eosin for histopathology and with rabbit polyclonal antiserum against BPV (catalogue number 2160100370, Quartett) for immunohistochemistry. The neoplastic mass consisted of squamous epithelial cells with cellular atypia, irregular papillary proliferation and orthokeratotic hyperkeratosis, but no invasion of the underlying stroma (Fig. 1a). Pale basophilic intranuclear inclusion bodies were observed infrequently in epithelial cells; the inclusion bodies were strongly positive for BPV antigens by immunohistochemistry (Fig. 1b).

The sequence of the 428 bp FAP59/FAP64 fragment was used to design a pair of specific primers (BPV10seq-1F/1R; see Appendix A: Supplementary Table 1) to amplify the remaining part of the genome using the LA Taq kit (Takara). A gel-purified PCR product representing the full genome was sequenced using the primer-walking method; the RCA product was sequenced directly and three whole genome clones derived from four partially-overlapping fragments cloned in pMD20-T were also sequenced (see Appendix A: Supplementary Table 2).

The sequence of the whole genome of BPV-10 MY-P55 had 7270 bp (GenBank KF017607), shared 99.4% nucleotide identity with BPV-10 AB331651 and had the same genome organisation. However, it had a 129 bp deletion in the early region E1 ORF (Fig. 2), which did not change the coding frame, but caused the loss of 43 amino acids. The L1 ORF of MY-P55 shared 99.5% nucleotide identity with AB331651. According to the guidelines for PV nomenclature published by the International Papillomavirus Workshop of the International Committee on Taxonomy of Viruses (de Villiers et al., 2004), MY-P55 was identified as a variant strain of BPV-10.

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[1] See: http://www.ncbi.nlm.nih.gov/blast.

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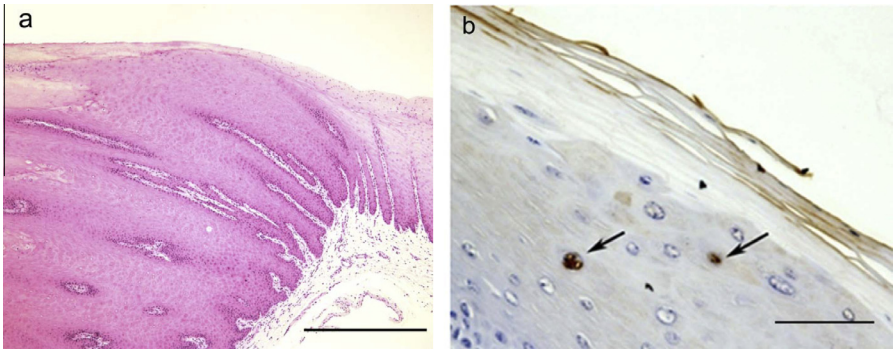

Fig. 1. Microscopic features of the papilloma (MY-P55) from the tongue of a cow. (a) Histopathology showing papillary proliferation of squamous epithelial cells. Haematoxylin and eosin. Bar = 700 μm. (b) Immunohistochemistry for papillomavirus demonstrates viral antigens (arrows) in intranuclear inclusion bodies. Bar = 100 μm.

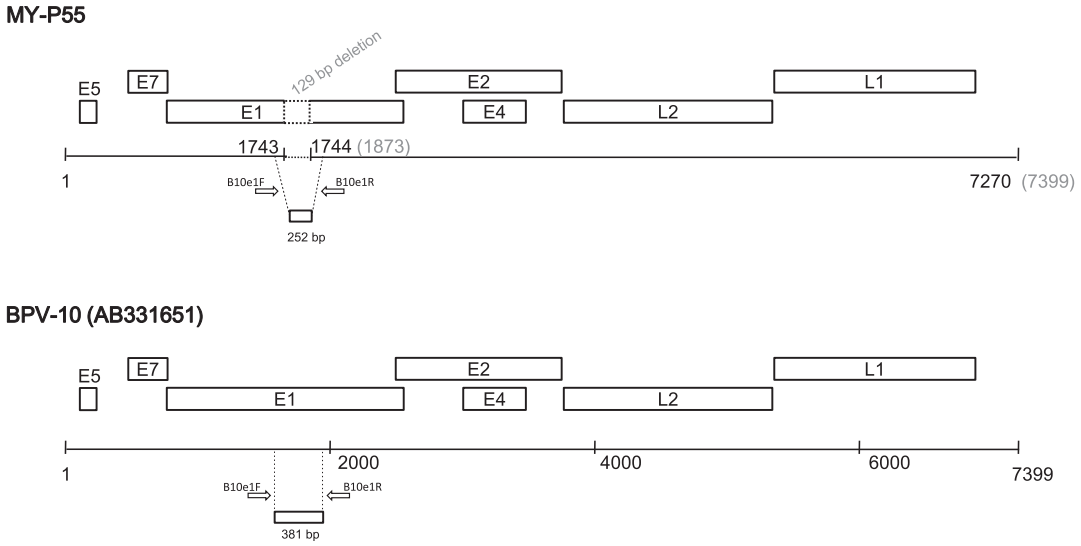

Fig. 2. Genomic map of BPV-10 MY-P55 and AB331651. The open reading frames (ORFs) are depicted as rectangles. The deletion in MY-P55 is indicated by a broken line. Arabic numerals indicate the nucleotide numbers of genomes (base pairs). The primer set, which was used to confirm the deletion in MY-P55, is shown by arrows, while the amplicons are depicted as rectangles and the length of amplicons is indicated below.

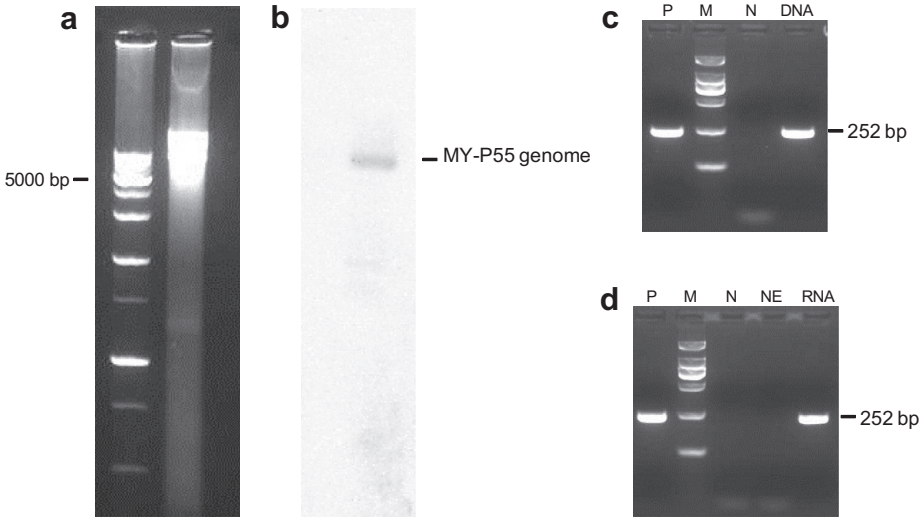

Fig. 3. Southern blot analysis, PCR and reverse transcriptase (RT)-PCR for detection of BPV-10 MY-P55 with a genomic deletion. (a) Agarose gel electrophoresis of total DNA extracted from the BPV-10 MY-P55-infected tongue sample. Ethidium bromide staining. (b) A single band was detected on Southern blot analysis, with no evidence of non-deleted BPV in the papilloma sample. (c) PCR across the deleted segment of genomic DNA; a single band was generated. (d) RT-PCR for detection of mRNA. Lane P, positive control with PCR product amplified from the purified whole genome of MY-P55; Lane M, DNA marker ladder with 100, 250, 500, 750, 1000 and 2000 bp from top to bottom; Lane N, negative control in which the template was replaced with distilled water; Lane DNA, PCR product with total DNA as the template; Lane NE, without reverse transcription; template is total RNA treated with gDNA Eraser (Takara); Lane RNA, RT-PCR; template is cDNA transcribed from total RNA treated with gDNA Eraser.

Southern blot analysis was performed on DNA using the Amersham Gene Images AlkPhos Direct Labeling and Detection System (GE). The luminescent signal was acquired with a Cooled CCD Camera System (AE-6971 Light-Capture, ATTO). The gel-purified PCR product BPV10seq-1F/1R amplified from BPV-10 MY-P55 was used as a probe; a single band was detected (Fig. 3a and b), thus showing no evidence of non-deleted BPV in the sample.

PCR was performed to confirm the results of Southern blot analysis. One pair of specific primers (B10e1F/R; see Appendix A: Supplementary Table 2) was designed to amplify the 381 bp genome segment from nucleotides 1614–1994 of BPV-10 AB331651 and a 252 bp segment of MY-P55 (Fig. 2). PCR generated a single band of 252 bp (Fig. 3c) and sequencing confirmed that the PCR product was from BPV-10 with a genomic deletion.

Reverse transcriptase (RT)-PCR was performed to detect mRNA. Total RNA was treated and reverse transcribed using a PrimeScript RT reagent kit with gDNA Eraser (Takara). PCR was also performed without reverse transcription to confirm that genomic DNA had been eliminated after treatment with gDNA Eraser. The RT-PCR product was sequenced after separation by gel electrophoresis. BPV-10 mRNA with the expected deletion was detected in the papilloma sample (Fig. 3d), whereas mRNA transcribed from non-deleted BPV-10 was not detected. In a study of human PVs (HPVs), deletions in the L1 and/or L2 ORFs of HPV-5 and HPV-8 were associated with malignant but not benign tumours in the same patient (Deau et al., 1991).

Previously, we detected both complete and deleted genomes of BPV-12 in a papilloma from the tongue of a cow (Dong et al., 2013). In the present study, BPV-10 was demonstrated in a mucosal papilloma from the tongue of a cow, whereas previously this BPV type has only been associated with cutaneous papillomas in cattle. This suggests that BPV-10 does not have strict tissue specificity. The genome of this BPV-10 strain had a 129 bp deletion in the E1 ORF, whereas non-deleted BPV-10 was not detected in the same sample.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tvjl.2013.11.016.

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