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Short communication

Identification of unreported putative new bovine papillomavirus types in Brazilian cattle herds

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Abstract

The amplification by degenerate primers FAP59/FAP64 and sequencing allowed the detection of 15 putative new BPV types in cutaneous warts as well as in healthy skin. Four of these isolates were recently recognized as new BPV types (BPV-7, -8, -9, and -10) after determination of their complete genome sequences. In Brazil, investigations involving the definition of BPV types present in skin warts are still rare. The aim of the current study was to identify the BPV types associated with cutaneous papillomatosis observed in Brazilian cattle herds. Twenty-two cutaneous papilloma specimens were submitted to PCR assay employing the FAP primer pair. All PCR products with approximately 480 bp were submitted to direct sequencing. Cloning was performed for the amplicons which prior analysis revealed as putative new BPV types. From 16 cutaneous lesions, BPV-1, -2, and -6 were identified in two, six, and eight papilloma specimens, respectively. In addition, four putative new BPV types were identified in other six skin warts, and then designated as BPV/BR-UEL2 to -5. The detection of the BPV-1, -2, and -6 types in skin wart specimens supports the existence of these BPV types throughout the Brazilian cattle herd. In addition, the identification of four putative new BPV types is the first report of the presence of different BPV types in the American continent.

Keywords: Cattle; Cutaneous papillomatosis; Bovine papillomavirus; Phylogenetic analysis

1. Introduction

Papillomaviruses (PVs) are a diverse group of small, non-enveloped, and double-stranded circular DNA viruses that occur in a broad range of distantly related animal species such as birds, human beings,

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and bovines (Antonsson and Hansson, 2002; de Villiers et al., 2004).

While more than a hundred human papillomavirus (HPV) types have been detected from both benign and malignant lesions in humans, only six BPV types had been characterized before 1984 (Pfister et al., 1979; Campo et al., 1980, 1981; Campo and Coggins, 1982; Chen et al., 1982; Jarrett et al., 1984). However, the use of PCR assays with degenerate primers that amplify relatively conserved regions in the L1 gene, followed by sequencing, has allowed the identification of several PV types in human and other animal hosts.

The amplification by degenerate primers FAP59/FAP64 and sequencing enabled the detection of 15 putative new BPV types in teat skin warts as well as in healthy teat skin of cattle from Japan and Sweden (Forslund et al., 1999; Antonsson and Hansson, 2002; Ogawa et al., 2004). After characterization of their complete genome sequences, four of these Japanese isolates were recently recognized as new BPV types (BPV-7, -8, -9, and -10) (Ogawa et al., 2007; Tomita et al., 2007; Hatama et al., 2008).

At present, the *Papillomaviridae* family is formed by 18 genera (*Alphapapillomavirus* to *Sigmapapillomavirus*), being such classification based on nucleotide sequence diversity verified in the L1 open reading frame (ORF) (de Villiers et al., 2004). The BPV types are classified in four different genera, *Deltapapillomavirus* (BPV-1 and -2), *Xipapillomavirus* (BPV-3, -4, -6, -9, and -10), *Epsilonpapillomavirus* (BPV-5 and -8), and a yet unassigned PV genus (BPV-7) (de Villiers et al., 2004; Ogawa et al., 2007; Tomita et al., 2007; Hatama et al., 2008).

In Brazil, the association between the BPV infections and the occurrence of cutaneous papillomatosis, chronic enzootic haematuria, and cancer of upper gastrointestinal tract, has been suggested in cattle. Since late-1990s, only BPV-1 and BPV-2 had been described using specific primers in different clinical specimens (Santos et al., 1998; Freitas et al., 2003; Wosiacki et al., 2005, 2006). However, in recent investigations, the use of FAP primer pair also enabled the identification of BPV-1, -2, -6, and -8 in skin warts of cattle from Parana state of Brazil (Claus et al., 2007, submitted for publication).

The aim of the current study was to identify the BPV types associated with cutaneous papillomatosis observed in Brazilian cattle herds.

2. Materials and methods

2.1. Papilloma specimens

Twenty-two cutaneous papilloma specimens were individually collected from diverse body sites of adult bovines. The animals were originated from dairy ($n = 2$) and beef ($n = 2$) cattle herds from northern region of Parana state, southern Brazil. Fragments from each papilloma specimen were ground in phosphate-buffered saline solution (PBS pH 7.2), and suspensions (10–20%, w/v) were centrifuged for 15 min at 3000 × *g* at 4 °C. Aliquots (250 μl) from supernatant were treated with lysis buffer [10 mM Tris; 1 mM EDTA; 0.5% Nonidet P40; 1% SDS; and 0.2 mg/ml proteinase K (Invitrogen, Life Technologies, Carlsbad, USA)]. After homogenization, samples were incubated at 56 °C for 30 min.

2.2. DNA extraction

For DNA extraction, a combination of phenol/chloroform/isoamyl alcohol and silica/guanidine isothiocyanate methods was carried out according to Alfieri et al. (2006). Briefly, fractions from each sample were treated with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1), homogenized and heated at 56 °C for 15 min (Sambrook and Russell, 2001). After centrifugation at 10,000 × *g* for 10 min, the aqueous phase was processed according to silica/guanidine isothiocyanate method (Boom et al., 1990). DNA was eluted in 50 μl of ultrapure (MilliQ®) sterile water and kept at −20 °C until use. Aliquots of ultrapure sterile water were included as negative control in the DNA extraction procedures.

2.3. PCR assay

The PCR assay was carried out using the primer pair FAP59 (forward: 5′-TAACWGTIGGICAYCCWTATT-3′) and FAP64 (reverse: 5′-CCWATATCWVHCATITCICCATC-3′) according to Forslund et al. (1999), with slight modifications (Claus et al., 2007). Reaction was performed using 5 μl of the extracted DNA and 45 μl of PCR-mix consisting of 1 μl (20 pmol) from each primer; 200 μM of each dNTP (Invitrogen, Life Technologies, Carlsbad, USA); 2.5 units of *Platinum Taq* DNA polymerase (Invitrogen,