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Phylogenetic classification and clinical aspects of a new putative *Deltapapillomavirus* associated with skin lesions in cattle

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ABSTRACT. Bovine papillomaviruses (BPVs) are recognized as causal agents of benign and malignant tumors in cattle. Thirteen types of BPVs have already been described and classified into 3 distinct genera. Divergences in the nucleotide sequence of the L1 gene are used to identify new viral types through the employment of PCR assays with degenerated primers. In the present study, a method for identifying BPVs based on PCR-RFLP and DNA sequencing allowed the identification of a new putative *Deltapapillomavirus*, designated JN/3SP (JQ280500.1). The analysis of the L1 gene showed that this

strain was most closely related to the BPVs -1, -2, -13 , and OaPV1 (71-73% genetic similarity). In this study, we describe the detection of this new putative *Deltapapillomavirus* type and verify its phylogenetic position within the genus.

Key words: Bovine papillomavirus; New putative *Deltapapillomavirus*; Phylogenetics; PCR-RFLP; Sequencing

INTRODUCTION

Papillomaviruses (PVs) are oncogenic viruses, with a double-stranded circular DNA genome of approximately 8 kb. The family Papillomaviridae is currently divided into 29 genera, formed by 190 papillomavirus types (de Villiers et al., 2004; Bernard et al., 2010), according to their genomic organization (Chan et al., 1995). Species from different PV genera show less than 60% identity with the L1 gene nucleotide sequence, whereas species within a genus share 60-70% identify. A PV isolate is recognized as being a new type of isolate if the complete genome has been cloned, and the DNA sequence of the L1 gene differs by more than 10% from the closest known PV type. Differences of 2-10% homology define a subtype, while differences of <2% homology define a variant (de Villiers et al., 2004; Bernard et al., 2010).

Bovine papillomaviruses (BPVs) are recognized as causal agents of benign and malignant tumors in cattle, such as cutaneous papillomas, fibropapillomas, urinary bladder, and esophageal cancer (Stocco dos Santos et al., 1998; Campo, 2003). To date, 13 types of BPVs have been characterized and classified into 3 distinct genera; namely, Delta, Epsilon, and Xi. In addition, each BPV has been associated with type-specific lesions (Borzacchiello and Roperto, 2008). For example, the BPVs -1, -2, and -13 are classified in the *Deltapapillomavirus* genus. These viruses induce fibropapillomas, which are associated with the recruitment of sub-epithelial fibroblasts (Lunardi et al., 2013b). BPV-1 is generally associated with lesions of the teats and udder in cattle (Jarrett et al., 1984; Hatama et al., 2008). This type of virus is also associated with fibropapillomas in the animal penis, leading to necrosis, and loss of reproductive function (Gardiner et al., 2008). BPV-2 is a causal agent of malignant bladder tumors, in addition to cutaneous fibropapillomas (Jarrett et al., 1984). Both types of viruses have also been detected in the peripheral blood and reproductive tissues samples of cattle, indicating vertical transmission (Carvalho et al., 2003; Roperto et al., 2008; Diniz et al., 2009). Recently, the genome of a new BPV type (BPV-13) was fully sequenced and identified as a Delta-BPV (Lunardi et al., 2013b). It has been attributed to the ability of bovine *Deltapapillomavirus* to infect different host species, causing the equine sarcoid (Nasir and Reid, 1999; Nasir and Campo, 2008, Lunardi et al., 2013a). A greater number of BPV types (-3, -4, -6, -9, -10, -11, and -12) belong to the *Xipapillomavirus* genus, and are considered exclusively epitheliotropic viruses, inducing the formation of the so-called "true papillomas" (Jarrett et al., 1984; Hatama et al., 2011; Zhu et al., 2012). In comparison, the BPVs -5 and -8 have the potential to induce fibropapillomas and true papillomas, being classified into a 3rd genus, the *Epsilonpapillomavirus* genus (Tomita et al., 2007). BPV-7 is classified separately, to date (Ogawa et al., 2007).

The genome of most papillomavirus contains 8 genes divided into 3 regions; specifically, 1) early region, which encodes the transforming (E5, E6, and E7) and replication (E1 and E2) of proteins; 2) long control region (LCR), which contains the origin of replication and the binding sites of multiple transcription factors; and 3) late region, which encodes the major

and minor proteins of the capsid (L1 and L2) (Zheng and Baker, 2006). The L1 gene is important to papillomavirus taxonomy, because it is the most conserved gene within the PV genome and, therefore, has been used to identify new viral types (de Villiers et al., 2004; Bernard et al., 2010; Bernard, 2013).

The use of PCR assays with degenerated primers, followed by sequencing, has facilitated the identification of several PV types in human and other animal hosts, including BPVs in bovines (Hatama et al., 2011; Zhu et al., 2012). The 13 described BPV types, as well as other new putative PVs, have been confirmed by molecular biological methods, based on the nucleotide sequence diversity of the L1 gene (Ogawa et al., 2007; Claus et al., 2008; Hatama et al., 2011; Zhu et al., 2012). PCR assays using degenerated primers that amplify partial fragments of the L1 gene, followed by sequencing, have demonstrated the presence of numerous new putative BPV types in cattle herds from different geographical regions (Silva et al., 2010). Investigations using the degenerated primers FAP59/FAP64 and MY09/MY11 identified 16 new putative BPV types (FAP59/FAP64: BAA-1 to -4, BAPV-3 to -5, BAPV-7 to -10, BAPV-11MY, and BPV/BRUEL-2, -3, -5; MY09/MY11: BAA-1 to -4, BAPV-2 to -5, BAPV-7 to -10, BAPPV11MY, and BPV/BR-UEL2 to 5) from healthy skin swabs or cutaneous warts of herds in Sweden, Japan, and Brazil (Ogawa et al., 2004; Claus et al., 2008; Hatama et al., 2008).

Different BPV types have already been detected in Brazil. Reports available from different regions of the country indicate a significant diversity of viral types in Brazil, implying significant economic losses due to the associated diseases (Roperto et al., 2008; Lindsey et al., 2009; Lunardi et al., 2010). Of note, the putative BPV type BR- UEL- 4 under accession No. EU293540, which is presently designated as BPV-13, was identified from a bovine skin wart and also in horse sarcoid tumor sample that were typically associated with the *Deltapapillomavirus* infection (Claus et al., 2008; Silva et al., 2010; Lunardi et al., 2013a). Here, we identify another new putative PV, designated JN/3SP (accession No. JQ280500.1), and determined its phylogenetic position into the *Deltapapillomavirus* genus. This description includes histological data of the lesions analyzed.

MATERIAL AND METHODS

Ethics statement

The protocols used in this study were approved by the Ethics Committee in Research from the Universidade Federal de São Paulo, Brazil, (Protocol No. 1829/09) assigned by the President of this committee. All efforts were made to minimize any suffering to the animals.

Animal selection

Four 6-year old Simmental cows, with disseminated cutaneous papillomatosis were selected. Wart fragments were collected in PBS buffer for DNA extraction.

Histopathological analysis

A fraction of all collected papilloma samples was fixed in 10% buffered formalin, embedded in paraffin, sliced into 5 μm sections, and stained with hematoxylin and eosin (HE).

DNA extraction and PCR

A fragment of each sample was subjected to DNA extraction, using a tissue and cells genomic Prep Mini Spin Kit (GE Healthcare, Buckinghamshire, UK). The quality of the DNA samples obtained was verified using the polymerase chain reaction (PCR) technique for the amplification of a 450 base pair (bp) fragment of the bovine β-globin, according to Yaguiu et al. (2008). Viral molecular identification was performed using the degenerate set of primers FAP. An approximately 478-bp segment of the L1 gene was amplified using the following primer sequences: forward: FAP59 (5'-TAA CWG TIG GIC AYC CWT ATT-3'); reverse: FAP64 (5'-CCW ATA TCW VHC ATI TCI CCA TC-3'). The PCR was performed with slight modifications of a previously described protocol (Ogawa et al., 2004; Yaguiu et al., 2008). Specifically, the amplification reactions were performed in a Corbett CG1-96 thermocycler (Corbett Life Science, Sydney, Australia) with a GoTaq Master Mix (Promega, Madison, Wisconsin, USA) under the following conditions: 5 min at 95°C, followed by 35 cycles of 1.5 min at 95°C, 2 min at 52°C, and 1.5 min at 72°C, and a final extension step of 5 min at 72°C. The PCR products were analyzed on 2% agarose gel electrophoresis stained with GelRed™ (Nucleic Acid Gel Stain; Biotium, Hayward, CA, USA), and visualized under ultra-violet (UV) light.

Restriction analysis

The L1 FAP segment digestion profiles of BPVs-1 to -13 were generated by NEBcutter 2.0 (Carvalho RF, personal communication) from all L1 complete nucleotide sequences available in the Genbank (http://www.ncbi.nlm.nih.gov/genbank/). Digestion reactions for RFLP profiling were performed with the *Dde*I enzyme (Table 1), following manufacturer protocols (New England Biolabs, Ipswich, USA). Cloned BPV-2 genome was used as positive controls. The PCR-RFLP products were analyzed on 2% agarose gel electrophoresis in TAE (*tris*/*acetate*/*EDTA*) buffer, stained with GelRed™ (Nucleic Acid Gel Stain), and visualized under UV light. PCR samples with abnormal or inconclusive RFLP patterns were subjected to repeat sequencing.

Table 1. RFLP profiles for *Dde*I restriction enzyme of the BPVs-1 to -13.

	BPV-1	BPV-2	BPV-3	BPV-4	BPV-5	BPV-6	BPV-7	BPV-8	BPV-9	BPV-10	BPV-11	BPV-12	BPV-13
L1 FAP fragment	475 bp	475 bp	473 bp	469 bp	469 bp	472 bp	484 bp	469 bp	469 bp	472 bp	475 bp	469 bp	475 bp
Fragments sized obtained with *Dde*I	264 159	316 159	319 154	324 145	469 321	151 403	81 469	316 153	319 90	63 55	420 118	351 316	109 50

Cloning and sequencing

The purified amplification products were cloned in TOPO TA cloning vector (pCR™ 4-TOPO™ Vetor-Invitrogen, California, USA). Recombinant plasmids were used to transform competent DH5α *E. coli* cells, being subsequently recovered for sequencing reactions. Three independent sequencing reactions were created for each cloned PCR fragment in an ABI377 PRISM Genetic Analyzer (Life Applied Biosystems, Foster City, California, USA). The quality of DNA sequences was checked, and overlapping fragments were assembled using the

BioEdit package software 7.0.9.0 (Hall, 1999). Assembled sequences with high quality were aligned using ClustalX1.83 with default gap penalties (Thompson et al., 1994).

Phylogenetic analysis

Homology analyses were performed with the NCBI database and BLAST (Altschul et al., 1997). Nucleotide and amino acid sequences from other BPV types, as well as from HPV-16 and other PVs, were retrieved from the GenBank for comparison with the sequences obtained here (JQ280500.1). The BioEdit software was used to identify the equivalent amino acid sequences. The sequenced dataset was analyzed with a Bayesian Markov-chain Monte Carlo (MCMC) approach (Yang and Rannala, 1997), using Mr Bayes 3.2 (Ronquist et al., 2012). The search for the sequence data relied on the GTR + Γ model, with the number of gamma categories being set to 4 (Yang, 1993). Bayesian runs started from independent random starting trees and were repeated 4 times. Markov chain Monte Carlo runs extended for 10 million generations, with trees being sampled every 1000th generation.

RESULTS

The FAP PCR products were amplified from the 4 examined papilloma samples that had the expected molecular size. RFLP and subsequent DNA sequencing analysis identified BPV-2 in 1 sample (located in the head) (Figure 1) and, more relevantly, as yet unknown BPV isolate was identified in the other 3 samples (1 located in the head and 2 in the udder). This new putative type (JN/3SP) was genetically similar (Table 2) to *Delta*BPVs, and to another *Deltapapillomavirus* type, OaPV1, which is known to infect ovines (*Ovisaries*).

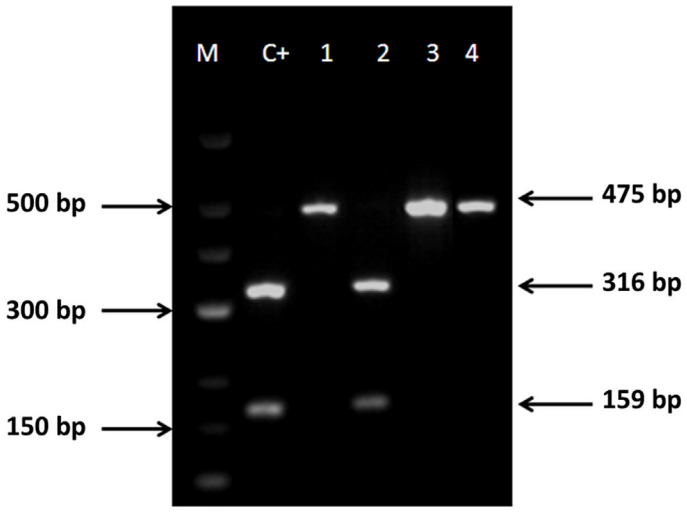

Figure 1. FAP amplified product digestion: 2% agarose gel electrophoresis. *Lanes M* = GeneRuler Low Range DNA Ladder™; C+ = positive control (cloned BPV-2 genome). *Lanes 1*, *3*, and *4* = undigested PCR sample; *Lane 2* = PCR digested sample (BPV-2). All samples correspond to the amplified products with generic primers FAP59/FAP64, digested with *Dde*I.

Table 2. Identity matrix indicating the similarity between different PV type.

Genera	*Deltapapillomavirus*										*Epsilonpapillomavirus*		*Xipapillomavirus*						Unsigned	*Dyodeltapapillomavirus*	*Alphapapillomavirus*
Scient. abbrev.	BPV-1	BPV-2	BPV-13	JN03SP	AaPV1	CcaPV1	OaPV1	OaPV2	OvPV1	RtPV1	BPV-5	BPV-8	BPV-3	BPV-4	BPV-6	BPV-9	BPV-10	BPV-11	BPV-12	BPV-7	SsPV1	HPV-16
BPV-1	ID	0.866	0.896	0.721	0.686	0.686	0.648	0.66	0.708	0.708	0.669	0.639	0.57	0.57	0.605	0.579	0.622	0.583	0.562	0.467	0.529	0.527
BPV-2	0.866	ID	0.901	0.721	0.69	0.686	0.66	0.656	0.665	0.665	0.643	0.648	0.592	0.566	0.613	0.583	0.6	0.579	0.57	0.497	0.516	0.545
BPV-13	0.896	0.901	ID	0.729	0.669	0.69	0.635	0.643	0.669	0.669	0.63	0.639	0.579	0.557	0.613	0.557	0.613	0.579	0.587	0.472	0.516	0.527
JN03SP	0.721	0.721	0.729	ID	0.669	0.703	0.712	0.695	0.673	0.673	0.656	0.652	0.583	0.587	0.545	0.549	0.583	0.553	0.587	0.532	0.546	0.562
AaPV1	0.686	0.69	0.669	0.669	ID	0.695	0.63	0.652	0.742	0.742	0.652	0.622	0.575	0.549	0.519	0.566	0.545	0.545	0.519	0.484	0.512	0.51
CcaPV1	0.686	0.686	0.69	0.703	0.695	ID	0.665	0.678	0.69	0.69	0.639	0.63	0.592	0.579	0.54	0.583	0.562	0.562	0.536	0.523	0.555	0.57
OaPV1	0.648	0.66	0.635	0.712	0.63	0.665	ID	0.793	0.643	0.643	0.6	0.613	0.536	0.54	0.549	0.54	0.575	0.553	0.562	0.489	0.563	0.566
OaPV2	0.66	0.656	0.643	0.695	0.652	0.678	0.793	ID	0.656	0.656	0.63	0.66	0.566	0.566	0.553	0.592	0.545	0.566	0.549	0.467	0.559	0.562
OvPV1	0.708	0.665	0.669	0.673	0.742	0.69	0.643	0.656	ID	1	0.639	0.605	0.575	0.57	0.54	0.553	0.562	0.557	0.54	0.484	0.559	0.549
RtPV1	0.708	0.665	0.669	0.673	0.742	0.69	0.643	0.656	1	ID	0.639	0.605	0.575	0.57	0.54	0.553	0.562	0.557	0.54	0.484	0.559	0.549
BPV-5	0.669	0.643	0.63	0.656	0.652	0.639	0.6	0.63	0.639	0.639	ID	0.759	0.54	0.579	0.553	0.587	0.553	0.557	0.566	0.515	0.58	0.583
BPV-8	0.639	0.648	0.639	0.652	0.622	0.63	0.613	0.66	0.605	0.605	0.759	ID	0.557	0.545	0.583	0.587	0.575	0.579	0.592	0.532	0.538	0.583
BPV-3	0.57	0.592	0.579	0.583	0.575	0.592	0.536	0.566	0.575	0.575	0.54	0.557	ID	0.716	0.729	0.768	0.69	0.716	0.682	0.63	0.563	0.523
BPV-4	0.57	0.566	0.557	0.587	0.549	0.579	0.54	0.566	0.57	0.57	0.579	0.545	0.716	ID	0.699	0.761	0.708	0.791	0.686	0.6	0.567	0.553
BPV-6	0.605	0.613	0.613	0.545	0.519	0.54	0.549	0.553	0.54	0.54	0.553	0.583	0.729	0.699	ID	0.755	0.686	0.686	0.678	0.613	0.584	0.587
BPV-9	0.579	0.583	0.557	0.549	0.566	0.583	0.54	0.592	0.553	0.553	0.587	0.587	0.768	0.761	0.755	ID	0.744	0.761	0.649	0.619	0.58	0.553
BPV-10	0.622	0.6	0.613	0.583	0.545	0.562	0.575	0.545	0.562	0.562	0.553	0.575	0.69	0.708	0.686	0.744	ID	0.743	0.66	0.617	0.593	0.553
BPV-11	0.583	0.579	0.579	0.553	0.545	0.562	0.553	0.566	0.557	0.557	0.557	0.579	0.716	0.791	0.686	0.761	0.743	ID	0.7	0.604	0.542	0.54
BPV-12	0.562	0.57	0.587	0.587	0.519	0.536	0.562	0.549	0.54	0.54	0.566	0.592	0.682	0.686	0.678	0.649	0.66	0.7	ID	0.592	0.559	0.566
BPV-7	0.467	0.497	0.472	0.532	0.484	0.523	0.489	0.467	0.484	0.484	0.515	0.532	0.63	0.6	0.613	0.619	0.617	0.604	0.592	ID	0.555	0.575
SsPV1	0.529	0.516	0.516	0.546	0.512	0.555	0.563	0.559	0.559	0.559	0.58	0.538	0.563	0.567	0.584	0.58	0.593	0.542	0.559	0.555	ID	0.665
HPV-16	0.527	0.545	0.527	0.562	0.51	0.57	0.566	0.562	0.549	0.549	0.583	0.583	0.523	0.553	0.587	0.553	0.553	0.54	0.566	0.575	0.665	ID

The histopathology of the skin lesion fragments of the animals (2 samples from the head and 2 from udders) infected with BPV-2, and the new putative *Deltapapillomavirus* JN/3SP showed a marked increase in epidermal koilocytosis, as well as other common features for this type of viral infection, such as papillomatosis, hyperkeratosis, acanthosis, and mild inflammatory infiltrates in the dermis and epidermis. The histopathology of the skin lesions of the animals infected with the putative new virus type showed a marked increase in epidermal koilocytosis, in addition to other common features of viral infections (Figure 2).

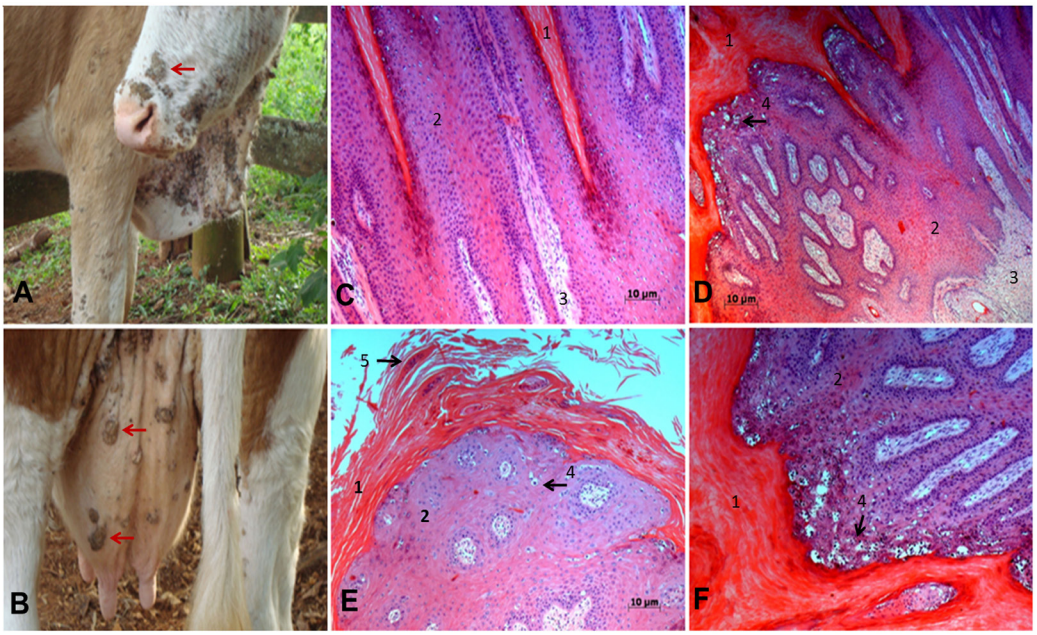

Figure 2. Animal with papillomatosis and histopathological evaluation of skin lesion fragments. **A.** and **B.** Cutaneous papillomatosis on the face and udder. **C.** and **E.** Skin lesion infected with BPV-2 (5X and 10X objectives). **D.** and **F.** Skin lesion infected with new putative bovine (JN/3SP) (5X and 10X). Histopathological preparations with stained hematoxylin/eosin (HE): 1 = hyperkeratosis; 2 = acanthosis; 3 = inflammatory infiltrate in the dermis; 4 = koilocytosis; 5 = parakeratosis; Bar = 10 µm.

The partial L1 sequence (431 bp) of JN/3SP was compared to the L1 nucleotide sequences of all BPV types (1-13), as well as the L1 sequences of the other *Deltapapillomavirus* from different species (Table 3). Another comparison was done with other papillomavirus phylogenetically more distant, such as HPV-16 (*Alfapapillomavirus*) and SsPV1 (*Dyodeltapapillomavirus*) (Figure 3). Although the putative JN/3SP has been found in cattle, over 70% of the partial L1 gene showed similar genus *Ovis aries* Papillomavirus 1, *Deltapapillomavirus* (Table 2).

Table 3. Alphabetic listing of the nine *Deltapapillomavirus* of the different species.

Scientific abbreviation	Papillomavirus name	Genus (Phylogeny)	Species (common use species ICTV)	NCBI #	Reference
AaPV1	*Alces alces* Papillomavirus 1	*Deltapapillomavirus* (Delta-1)	*Alces alces Papillomavirus* 1	M15953	Ahola et al., 1986
BPV1	*Bos taurus* Papillomavirus 1	*Deltapapillomavirus* (Delta-4)	*Bos taurus Papillomavirus* 1	X02346	Chen et al., 1982
BPV2	*Bos taurus* Papillomavirus 2	*Deltapapillomavirus* (Delta-4)	*Bos taurus Papillomavirus* 1	M20219	Groff et al., 1986*
BPV-13	*Bos taurus* Papillomavirus 2	*Deltapapillomavirus* (Delta-4)	*Bos taurus Papillomavirus* 1	JQ798171	Lunardi et al., 2013b
CcaPV1	*Capreolus capreolus* Papillomavirus 1	*Deltapapillomavirus* (Delta-5)	*Capreolus capreolus Papillomavirus* 1	EF680235	Erdélyi et al., 2008
OaPV1	*Ovis aries* Papillomavirus 1	*Deltapapillomavirus* (Delta-3)	*Ovis aries Papillomavirus* 1	U83594	Karlis et al., 2000*
OaPV2	*Ovis aries* Papillomavirus 2	*Deltapapillomavirus* (Delta-3)	*Ovis aries Papillomavirus* 1	U83595	Karlis et al., 2000*
OvPV1	*Odocoileus virginianus* Papillomavirus 1	*Deltapapillomavirus* (Delta-2)	*Odocoileus virginianus Papillomavirus* 1	M11910	Groff et al., 1986*
RtPV1	*Rangifer tarandus* Papillomavirus 1	*Deltapapillomavirus* (Delta-1)	*Alces alces Papillomavirus* 1	AF443292	Burk et al., 2001*

*Direct submission.

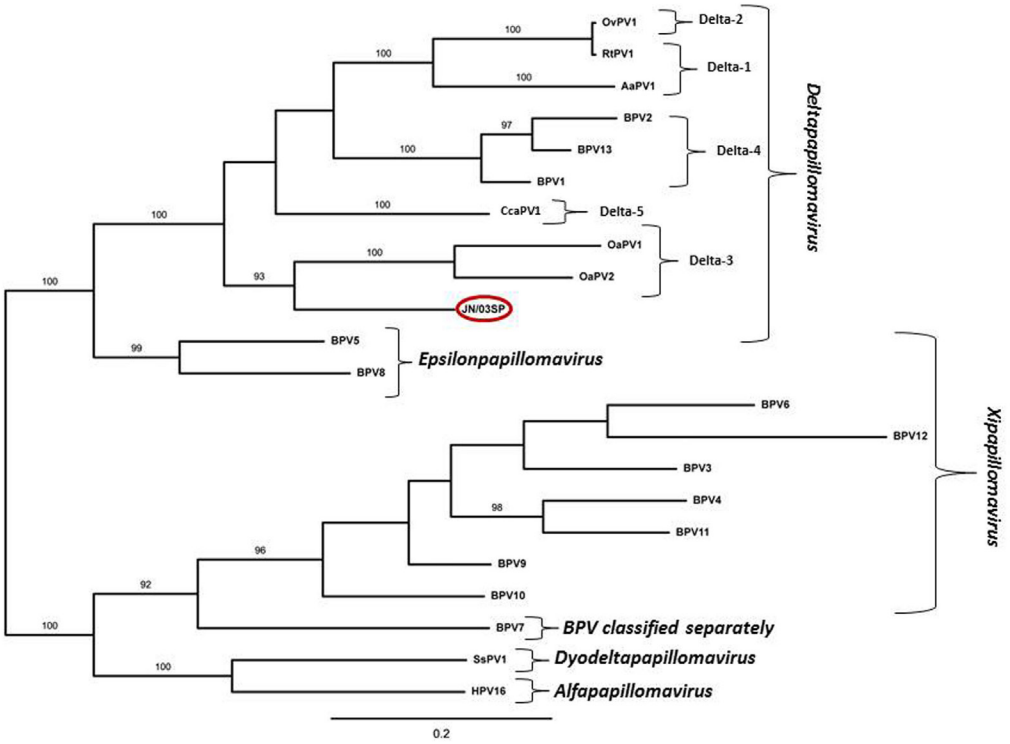

Figure 3. Phylogenetic Bayesian tree of FAP L1 ORF, including the BPV- JN/3SP (GenBank accession No. JQ280500.1). The tree is divided into the previously determined genera *Deltapapillomavirus* (BPV-1,-2 and -13, OvPV1, RtPV1, AaPV1, BpPV1, CcPV1, OaPV1, and OaPV2), *Epsilonpapillomavirus* (BPV-5 and -8), *Xipapillomavirus* (BPV-3, -4, -6, -9, -10, -11, -12), the undesignated BPV-7 and HPV-16. Numbers at nodes are Bayesian posterior probabilities. Scale bar represents the number of estimated changes per position for a unit of branch length. The following reference sequences were used: BPV-1 (X02346), BPV-2 (PPB2CG), BPV-3 (AJ620207), BPV-4 (X05817), BPV-5 (AJ620206), BPV-6 (AJ620208), BPV-7 (DQ217793), BPV-8 (DQ098913), BPV-9 (AB331650), BPV-10 (AB331651), BPV-11 (AB543507), BPV-12 (JF834523), BPV-13 (JQ798171), UEL-2, -3 and -5 (EU293538, EU293539, and EU293541, respectively), HPV-16 (K02718), AaPV1 (M15953), CcaPV1 (EF680235), OaPV1 (U83594), OaPV2 (U83595), OvPV1 (M11910), RtPV1 (AF443292), SsPV1 (EF395818).

DISCUSSION

The FAP59/64 set was originally designed according to 2 relatively conserved regions of the L1 gene, and facilitated the characterization of a large diversity of HPV types (Forslund et al., 1999). One of the first descriptions of putative new types in bovines employing generic primers was published in 2002 (Antonsson and Hansson, 2002). Ogawa et al. (2004) reported the identification of authentic and putative BPV types in both lesions and healthy tissue. The authors also found that the FAP59/64 generic primer was more efficient at detecting PV compared to the MY09/11 primer set. Hence, the identification of the new putative JN/03SP was carried out using FAP. These primers enable us to suggest a new HPV type, and to test the effectiveness of RFLP techniques for BPV screening and typing (Carvalho et al., 2003).

The Papilomavirus Nomenclature Committee (14th International Papillomavirus Conference, Quebec City, Quebec, Canada) specified that: "The isolated novel sequences were called putative new PV types instead of PV types, since PCR products represent only part of the L1 gene" (Antonsson and Hansson, 2002; de Villiers et al., 2004). Analysis of the FAP L1 ORF sequence has shown that the JN/3SP strain is closely related to BPV-1, -2, and -13 (72-73% genetic similarity). However it also showed 71% of similarity with OaPV1. Although it is known that most new types of PV shows less than 60% sequence identity to types from other genera, the percentage identity is only used as a general criteria to define the PV genus. For instance, the assignment of PV types to species and genera cannot be determined just by a computer algorithm, but requires curation, i.e., interpretation based on phylogeny, genome organization, biology, and pathogenicity (Bernard, 2013).

The phylogenetic analysis based on amino acid sequence alignments yielded a tree with a very similar topology to that based on nucleic acid sequence alignments (data not shown). The identification of this new putative as a Delta type was performed through a methodological approach that addresses the correlation between the viral identity and the histopathological findings associated with the infection. Hence, macroscopic and histological data indicate that the collected lesions have fibropapilloma characteristics. Overall, these data indicate that JN/3SP represents a new *Deltapapillomavirus*. Thus, JN/3SP could be considered as the 2nd Brazilian *Deltapapillomavirus* isolate, after the new putative BR-UEL-4, currently named BPV-13 (Claus et al., 2008; Lunardi et al., 2013b). Similar to BPV-13, JN/3SP was identified from warts on the different body parts (head and udder) of different livestock in this study, indicating its pathogenic potential to induce cutaneous lesions (Lunardi et al., 2013b).

Compared to other BPV genera, BPV *Deltapapillomavirus* has the ability to infect different host species, causing equine sarcoid (Nasir and Reid, 1999; Nasir and Campo, 2008, Lunardi et al., 2013a). Equine sarcoid is a common horse epithelial tumor, the etiology of which has been previously associated with BPV-1 and, less frequently, with BPV-2 (Claus et al., 2008; Nasir and Campo, 2008). To date, this ability to infect different host species has been attributed exclusively to BPV-1 and BPV-2 (Nasir and Reid, 1999; Nasir and Campo, 2008). However, Silva et al. (2010) identified BR-UEL-4 (BPV-13) from both skin papillomas of cows and equine sarcoid samples. This observation indicates that this new *Deltapapillomavirus* type might also be associated with the development of equine sarcoids as well (Lunardi et al., 2013a). This phenomenon could also be the case for JN/3SP; however, further studies are necessary to establish its potential to infect equine hosts, in addition to other potential livestock hosts.

Brazil has a high standing in the world market of bovine meat; yet, bovine papillomatosis is a persistent disease among Brazilian cattle. Therefore, this study aimed to obtain a better understanding about the clinical aspects related to BPV infection and its diagnosis. Hence, our findings are highly relevant for the development of new sanitary measures, including vaccines. At present, the typing of BPV is considered a laborious task, due to the presence of unknown viral types, in addition to co-infections in the infected samples that require the employment of cloning and DNA sequencing techniques. Our results provide evidence supporting the effectiveness of the PCR-RFLP/sequencing procedure for the detection and identification of new BPV types.

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