Molecular characterization of bovine *Deltapapillomavirus* (BPV1, 2, and 13) DNA in equine sarcoids[1]

Brígida K. de Alcântara[2], Amauri A. Alfieri[2]*, Selwyn A. Headley[3], Wagner B. Rodrigues[2], Rodrigo A.A. Otonel[2], Michele Lunardi[4] and Alice F. Alfieri[2]

ABSTRACT.- De Alcântara B.K., Alfieri A.A., Headley S.A., Rodrigues W.B., Otonel R.A.A., Lunardi M. & Alfieri A.F. 2015. **Molecular characterization of bovine *Deltapapillomavirus* (BPV1, 2, and 13) DNA in equine sarcoids.** *Pesquisa Veterinária Brasileira 35(5):431-436.* Laboratory of Animal Virology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, Campus Universitário, Cx. Postal 10011, Londrina, PR 86057-970, Brazil. E-mail: alfieri@uel.br

Sarcoids are fibroblastic lesions, which are considered as the most common skin tumors of horses; spontaneous regression rarely occurs. The bovine papillomavirus (BPV) types 1 and 2 may be involved in the pathogenesis of sarcoids, and probably the recently described BPV type (BPV13) might be associated with the pathogenesis of this lesion. This study characterized the DNA of BPVs in sarcoids from 15 horses from Brazil by analyzing 20 cutaneous lesions (12 recently collected; 8 from formalin-fixed paraffin-embedded (FFPE) tissues). Histopathology confirmed the proliferative lesions as sarcoids. Three PCRs were performed to amplify papillomavirus (PV) DNA. For screening, the primers IFNR2/IDNT2 were used to amplify a fragment of the PV L1 ORF. The second primer set was complementary to a common sequence of the E5L2 genomic region of BPV1, 2, and 13. The third primer pair (FAP59/FAP64) targeted a fragment of the PVs L1 ORF. The screening and E5L2 PCRs yielded amplicons in all samples evaluated. The FAP amplicons identified BPV1, 2, and 13 only from fresh tissue samples. The phylogenetic analyses of E5L2 resulted in the identification of BPV1, 2, and 13 in 14 (70%), 2 (10%), and 4 (20%) sarcoids, respectively. Two horses demonstrated multiple lesions: the sarcoids of one of these contained only BPV1 DNA and those of the other contained three types of bovine *Deltapapillomavirus* (BPV1, 2, and 13). This study confirmed the presence of BPV1, 2, and 13 DNA in equine sarcoids. Moreover, these findings represent the first description of three types of BPV diagnosed in the same horse, as well as the first confirmation of BPV1 and 2 in horses from Brazil.

INDEX TERMS: Papillomavirus, Bovine *Deltapapillomavirus,* BPV1, 2, and 13, DNA, equine sarcoids, horse, skin tumor.

RESUMO.- [**Caracterização molecular de DNA de *Deltapapillomavirus* bovino (BPV1, 2 e 13) em sarcoides equinos.**] Sarcoides são tumores fibroblásticos, considerados os tumores de pele mais comuns em pele de equinos e que raramente apresentam regressão espontânea. Papilomavírus bovino (BPV) tipos 1 e 2 são relacionados com a patogenia do sarcoide e, provavelmente, o BPV tipo 13 (BPV13), recentemente descrito, também pode estar associado com a formação dessa lesão. Neste estudo, 20 amostras de lesões cutâneas, sendo 12 constituídas por tecidos frescos e 8 amostras de tecido fixado em formalina e embebido em parafina, provenientes de 15 cavalos foram utilizadas para a identificação do DNA de BPV. A análise histopatológica (HE) confirmou todas as lesões como sarcoide. Para a amplificação do DNA de papilomavírus (PV) foram realizadas três reações de PCR. Como triagem, os *primers* IFNR2/IDNT2

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[2] Laboratório de Virologia Animal, Departamento de Medicina Veterinária Preventiva (DMVP), Universidade Estadual de Londrina (UEL), Rodovia Celso Garcia Cid, Campus Universitário, Cx. Postal 10011, Londrina, PR 86057-970, Brazil. *Corresponding author: alfieri@uel.br

[3] Laboratório de Patologia Animal, DMVP-UEL, Rodovia Celso Garcia Cid, Campus Universitário, Cx. Postal 10011, Londrina, PR.

[4] Laboratório de Microbiologia Veterinária, Hospital Veterinário Escola, Universidade de Cuiabá (Unic), Avenida Beira Rio 3100, Cuiabá, MT 78015-480, Brazil.

foram utilizados para amplificar um fragmento da ORF L1 do PV. O segundo par de *primers* utilizado é complementar a sequência dos genes E5 e L2 de BPVs 1, 2 e 13. O terceiro par de *primers* (FAP59/FAP64) utilizado tem o gene L1 como alvo. A primeira e a segunda PCRs permitiram amplificar produtos em todas as amostras avaliadas. Entretanto, na terceira reação, na qual foram utilizados os *primers* FAP, foi possível amplificar produtos com tamanho molecular esperado somente nas amostras constituídas por tecidos frescos. O sequenciamento de nucleotídeos e as análises filogenéticas realizadas nos fragmentos E5L2 resultaram na identificação de BPV1, 2 e 13 em 14 (70%), 2 (10%) e em 4 (20%) amostras de sarcoides, respectivamente. As amostras de sarcoides de um dos animais continha somente o DNA de BPV1. Entretanto, nas amostras provenientes do segundo cavalo foi possível identificar o DNA de três tipos de *Deltapapillomavirus* bovino (BPV1, 2 e 13) em lesões distintas. Este estudo ratifica a presença do DNA de BPV1, 2 e 13 em lesões de sarcoides em equinos, além de identificar três tipos de BPVs em um mesmo animal e descrever pela primeira vez no Brasil a presença de BPV1 e 2 nesse tipo de lesão.

TERMOS DE INDEXAÇÃO: Papilomavírus, *Deltapapillomavirus* bovino, BPV1, 2 e 13, DNA, sarcoide equino, cavalo, tumor de pele.

INTRODUCTION

Sarcoids are fibroblastic cutaneous lesions and the most frequently occurring skin tumors of equids; these proliferative growths can be observed in any anatomic location of the affected animal, while spontaneous regression rarely occurs (Nasir & Campo 2008). Moreover, the presence of sarcoids can significantly impact the health and welfare of affected horses due to the difficulties associated with therapy and frequent recurrence following surgical intervention (Stadler et al. 2011). In addition to horses, this tumor has been described in a wide variety of animals including zebras (Van Dyk et al. 2009), donkeys, mules (Reid et al. 1994), and captive tapirs (Kidney & Berrocal 2008).

Several studies have demonstrated that the bovine papillomavirus types 1 and 2 (BPV1, and 2) are involved in the pathogenesis of equine sarcoids (Otten et al. 1993, Bogaert et al. 2007, Marchetti et al. 2009, Hartl et al. 2011, Wobeser et al. 2012, Wilson et al. 2013). The episomal BPV DNA was detected within the fibroblastic nuclei (Amtmann et al. 1980), while the BPV1 DNA has been isolated from the epidermal layer of sarcoids (Brandt et al. 2011). Additionally, the BPV1 DNA was detected in a squamous cell carcinoma of one pony, demonstrating that BPV can be associated with other tumorous growths in horses (Kainzbauer et al. 2012). Moreover, since BPV DNA has been isolated from the normal skin of horses, BPV infection *per se* is probably not an adequate initiator of sarcoids (Bogaert et al. 2008). However, inflammatory cutaneous lesions can induce the expression of BPV oncoproteins, resulting in cell transformation (Borzacchiello et al. 2008, Corteggio et al. 2012, Wobeser et al. 2012).

Although sarcoids have similar histological characteristics, they have been classified into six (occult, verrucous, fibroblastic, nodular, mixed, and malevolent) different clinical lesions (Knottenbelt 2005). However, no association has been established between the BPV type and the proposed clinical classification (Nasir & Campo 2008). The lack of definite population data and the low disease reports have been the greatest obstacle to identify basic epidemiological features associated with equine sarcoids, since most descriptions are restricted to individual case reports or relatively small groups of affected animals (Reid et al. 1994, Brum et al. 2010, Wobeser et al. 2010).

Currently, thirteen BPV types have been identified in cattle and characterized in the genera *Deltapapillomavirus* (BPV1, 2, and 13); *Xipapillomavirus* (BPV3, 4, 6, 9, 10, 11, and 12); and *Epsilonpapillomavirus* (BPV5 and 8). While the BPV7 has not been designated into a papillomavirus (PV) genus (Bernard et al. 2010, Lunardi et al. 2013a). PCR assays using the degenerate primers, FAP59/FAP64 (Forslund et al. 1999), that have amplifed partial fragments of the L1 gene have been used to determine the presence of numerous putative new BPV types in cattle herds from diverse geographical regions (Ogawa et al. 2004, Claus et al. 2007, Claus et al. 2008). The most recent BPV type described was the BPV13 (GenBank accession number JQ798171) (Lunardi et al. 2013a), which was originally designated as BPV/BR-UEL4 (GenBank accession number EU293540) (Claus et al. 2008).

In Brazil, most investigations relating to equine sarcoids have been based on the clinical, epidemiological (Brum et al. 2010), and histopathological features of the disease (Souza et al. 2011). However, there are few molecular studies that indicate the presence of the DNA of this recent BPV type in sarcoids (Silva et al. 2010, Lunardi et al. 2013b). The present study investigated the occurrence of BPV DNA in equine sarcoids from horses of three geographical regions agreed of Brazil.

MATERIALS AND METHODS

Sarcoidal samples. Twenty cutaneous lesions samples of 15 horses, clinically classified as sarcoids, were used during this investigation of which 12 were recently collected by surgical procedure and eight were derived from formalin-fixed paraffin-embedded (FFPE) tissues. The freshly collected samples were mantained at 4°C until processed for molecular charactherization (less than 48 hours). The geographical distribution of the origin of all samples used in this study is given in Table 1; these were obtained from 15 horses within 13 herds from three geographical regions of Brazil: South (Santa Catarina, *n*=3 and Paraná *n*=4), Southeast (São Paulo, *n*=6), and Midwest (Federal District, *n*=2). The FFPE tissues were archival samples obtained from veterinary diagnostic laboratories located within the states of Santa Catarina (*n*=3) and São Paulo (*n*=5). All samples were collected during 2001 to 2012, with the oldest sample being a FFPE tissue. Two horses (#1 and 2; Herd A) from the state of Paraná developed sarcoids in different anatomical locations resulting in the evaluation of more than one lesions from these animals.

Clinical and histopathological evaluations. All freshly collected cutaneous lesions were clinically characterized (Knottenbelt 2005) and then fixed by immersion in 10% buffered formalin solution; these, as well as all FFPE blocks, were then routinely processed for histopathological evaluation.

DNA extraction. The DNA from recently collected samples was extracted by using the commercial kit Qiagen DNeasy blood

Table 1. The geographical distribution and clinical classification of equine sarcoids

Geographic regions*	Herd	Horse	Sample identification	Collecting year	Clinical classification
			Fresh tissue		
Paraná	A	1	Eq.Sar.BRA-1.1	2011	Fibroblastic
			Eq.Sarc.BRA-1.2	2011	Fibroblastic
			Eq.Sarc.BRA-1.3	2011	Mixed
	A	2	Eq.Sarc.BRA-2.1	2011	Fibroblastic
			Eq.Sarc.BRA-2.2	2011	Mixed
			Eq.Sarc.BRA-2.3	2011	Mixed
			Eq.Sarc.BRA-2.4	2011	Fibroblastic
	B	3	Eq.Sarc.BRA-3	2012	Fibroblastic
	C	4	Eq.Sarc.BRA-4	2012	Fibroblastic
São Paulo	D	5	Eq.Sarc.BRA-5	2012	Fibroblastic
Federal District	E	6	Eq.Sarc.BRA-6	2011	Mixed
	E	7	Eq.Sarc.BRA-7	2011	Fibroblastic
			Formalin fixed paraffin-embedded tissue		
São Paulo	F	8	Eq.Sarc.BRA-8	2001	Not provided
	G	9	Eq.Sarc.BRA-9	2006	-
	H	10	Eq.Sarc.BRA-10	2007	-
	I	11	Eq.Sarc.BRA-11	2009	-
	J	12	Eq.Sarc.BRA-12	2011	-
Santa Catarina	K	13	Eq.Sarc.BRA-13	2011	-
	L	14	Eq.Sarc.BRA-14	2011	-
	M	15	Eq.Sarc.BRA-15	2011	-

*Paraná and Santa Catarina states (Southern), São Paulo (Southeastern), and Federal District (Midwestern Brazil).

& tissue kit (Qiagen Sample & Assay Technologies, Hilden, Germany). Five 10 µm tissue sections were obtained from each FFPE block and used for DNA extraction; the microtome blade was changed between the collections of each sample to avoid cross-contamination. DNA extraction of FFPE tissue was performed with the Qiagen DNeasy FFPE Tissue Kit (Qiagen Sample & Assay Technologies, Hilden, Germany) in accordance with the manufacturer's instructions, with one modification: xylene was used at 56°C instead at room temperature, using chemical safety cabin. Aliquots of ultrapure sterile water were used as negative controls in all DNA extraction procedures.

PCR assays. In order to detect papillomavirus (PVs) DNA that might have been present in equine sarcoids, three different primer sets were used. The consensus primers IFNR-2/IDNT-2 were used for screening; this primer set amplifies a 102bp fragment of the PVs L1 gene (Kidney et al. 2001). The second primers used are complementary to a common sequence, with approximately 250bp, of the E5L2 open reading frame (ORF) of *Deltapapillomavirus*, according to Teifke et al. (2003) and Bogaert et al. (2005). The third PCR assay was done by using the primers FAP59/FAP64 (Forslund et al. 1999), whose target sequence is a 480bp fragment of the L1 gene of several PV strains. The FAP PCR was performed as described by Claus et al. (2007). A positive BPV control was included in all PCR assays. Aliquots of the PCR products were analyzed by electrophoresis in 2% agarose gel in TBE buffer pH 8.4 (89mM Tris; 89mM boric acid; 2mM EDTA) at constant voltage (90V) for approximately 45 min. The agarose gel was stained with ethidium bromide (0.5mg/ml) and visualized under ultraviolet light.

Sequence analysis. The PCR products were purified by using the illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare, Little Chalfont, UK), and quantified using the Quant-iT ™ dsDNA BR Assay kit[c] in the Qubit ™ Fluorometer (Invitrogen, Molecular Probes, Eugene, OR, USA). Direct sequencing was performed by using the BigDye Terminator v.3.1 Cycle Sequencing kit (Applied Biosystems, Carlsbad, USA) with the corresponding forward and reverse primers, in a 3500 Genetic Analyzer (Applied Biosystems, Carlsbad, USA), according to the manufacturer's instructions. The obtained sequences were examined with the PHRED software (http://asparagin.cenargen.embrapa.br/phph) for quality analysis of chromatogram readings. The sequences were accepted if the base quality was equal to or higher than 20. Consensus sequences were determined by CAP3 software (http://asparagin.cenargen.embrapa.br/cgi-bin/phph/cap3.pl) and sequence identity was verified with all sequences deposited in the GenBank using the BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

A multiple alignment was performed in CLUSTAL W (version 1.4) using MEGA package version 5.1 software and sequence identity matrix using BioEdit software version 7.0.8.0. Phylogenetic trees were obtained by the Maximum-likelihood method with the Kimura two parameter distance estimate (Kimura 1980), using MEGA package version 5.1 software. Statistical analyses of phylogenetic trees were determined by bootstrap method on 1,000 replicates.

RESULTS

Histopathological findings

The clinical classification of the recently collected cutaneous lesions is given in Table 1; eight sarcoids were classified as fibroblastic and four as mixed sarcoids. Multiple sarcoids in different anatomical locations occurred in horse #1 (three lesions) and #2 (four lesions); clinically, the sarcoids of these horses were classified as fibroblastic and mixed. The histopathological features of all sarcoids were similar (Table 2), being characterized by an extensive proliferating epidermis, which demonstrated orthokeratotic and/or parakeratotic hyperkeratosis, and invaded the underling dermis with several epithelial pegs. The dermis consisted of extensive sheets of proliferating fibroblasts arranged in

Table 2. Bovine papillomavirus types identified by PCR assays in equine sarcoids from three geographical regions of Brazil

Herd	Horse	Sample identification	Histopathological classification	BPV type[a]	
				L1 gene[b]	E5L2 gene[b]
		Fresh tissue			
A	1	Eq.Sar.BRA-1.1	Sarcoid	1	1
		Eq.Sarc.BRA-1.2	Sarcoid	1	1
		Eq.Sarc.BRA-1.3	Sarcoid	1	1
A	2	Eq.Sarc.BRA-2.1	Sarcoid	2	2
		Eq.Sarc.BRA-2.2	Sarcoid	13	13
		Eq.Sarc.BRA-2.3	Sarcoid	2	2
		Eq.Sarc.BRA-2.4	Sarcoid	1	1
B	3	Eq.Sarc.BRA-3	Sarcoid	13	13
C	4	Eq.Sarc.BRA-4	Sarcoid	-	1
D	5	Eq.Sarc.BRA-5	Sarcoid	13	13
E	6	Eq.Sarc.BRA-6	Sarcoid	1	1
E	7	Eq.Sarc.BRA-7	Sarcoid	1	1
		Formalin fixed paraffin-embedded tissues			
F	8	Eq.Sarc.BRA-8	Sarcoid	-	1
G	9	Eq.Sarc.BRA-9	Sarcoid	-	1
H	10	Eq.Sarc.BRA-10	Sarcoid	-	1
I	11	Eq.Sarc.BRA-11	Sarcoid	-	13
J	12	Eq.Sarc.BRA-12	Sarcoid	-	1
K	13	Eq.Sarc.BRA-13	Sarcoid	-	1
L	14	Eq.Sarc.BRA-14	Sarcoid	-	1
M	15	Eq.Sarc.BRA-15	Sarcoid	-	1

[a] BPV type according to highest similarity of identity matrix by BioEdit 7.0.8.0 software. [b]Result based on the alignment with the 341 bp fragment of BPV L1 gene and the 197 bp fragment of BPV E5L2 gene.

herringbone pattern or forming interlacing bundles. The neoplastic fibroblasts demonstrated discrete cellular and nuclear pleomorphism with reduced mitotic index.

BPV DNA detection and Phylogenetic analysis

A 102bp product was amplified from all 20 sarcoids using the consensus PV primers IFNR-2/IDNT-2. The amplicons of the expected length, approximately 250bp, were amplified from all DNA samples by PCR using the E5L2 primers. Sequencing of the E5L2 product resulted in the characterization of BPV1 (14/20; 70%), BPV2 (2/20; 10%), and BPV13 (4/20; 20%). However, the FAP PCR assays produced successful results only from 11 fresh tissue samples (11/20), and were characterized as BPV1 (6/11; 54.5%), BPV2 (2/11; 18.2%), and BPV13 (3/11; 27.3%). Nevertheless, the sequence results obtained during both PCR assays (E5L2 and FAP) were identical for these 11 samples (Table 2). The multiple sarcoids extracted from different anatomical regions of horse #1 contained BPV1 DNA. Alternatively, three sarcoids, from distinct anatomical locations of horse #2 contained DNA of three distinct BPVs types (BPV1, 2, and 13).

The nucleotide sequence identity for the E5L2 fragments varied between 99.4 to 100% for BPV1 (14 samples); was 97.8% for BPV2 (2 samples) and varied from 99.4 to 100% for BPV13 (4 samples). The nucleotide identity observed on the L1 gene fragment (341 bp) varied between 99.6 to 100% for BPV1 (6 samples), 98.8% to BPV2 (2 samples) and 99.6 to 100% to BPV13 (3 samples). The L1 fragment sequence of each BPV type was deposited at the GenBank database. The topology of the phylogenetic tree obtained for the L1 region was similar to that of E5L2 ORF (Fig.1 and 2). Consequnetly, the sequences from both regions confirmed that the BPVs isolates identified in this study clustered with BPV1, 2, and 13.

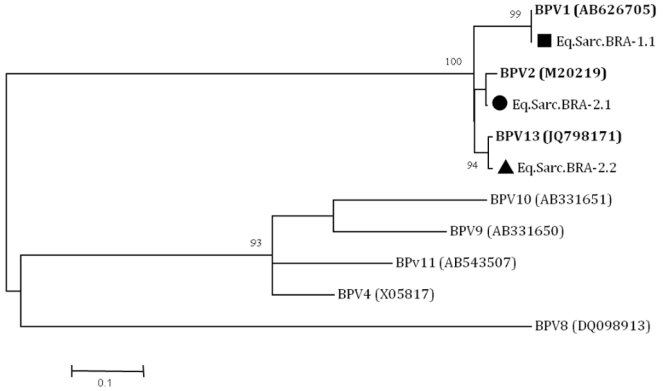

Fig.1. Phylogenetic tree obtained by the maximum-likelihood method based on the nucleotide sequences of a 197 bp fragment within PVs E5L2 gene. Bootstrap values are indicated when at least 50% as a percentage was obtained from 1,000 replications. The BPVs strains are marked according to the BPV cluster: BPV1 (square), BPV2 (circle), and BPV13 (triangle). The other samples, not included on figure, presented similar sequences. The E5L2 sequences were not submitted in GenBank because the fragment is shorter than 200bp in length. BPV sequence names are labelled as follows: BLAST identification and GenBank accession number. The scale bar represents a genetic distance of 0.1 substitutions per site.

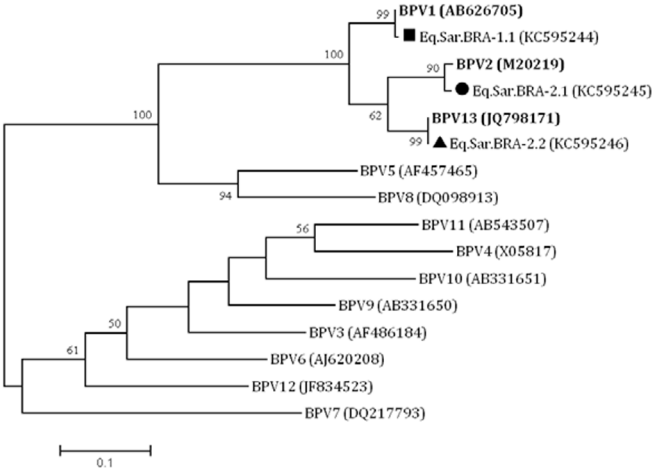

Fig.2. Phylogenetic tree obtained by the maximum-likelihood method based on the nucleotide sequences of a 341 bp fragment within BPV L1 gene, 5558 to 6013 nt of BPV1, 5725 to 6199 nt of BPV2 and 5745 to 6219 nt of BPV13. Bootstrap values are indicated when at least 50% as a percentage was obtained from 1,000 replications. The BPV strains from *Deltapapillomavirus* genus identified in equine sarcoids Eq.Sar.BRA-1.1, Eq.Sar.BRA-2.1, and Eq.Sar.BRA-2.2 are marked as square, circle, and triangle, respectively. The other samples, which were not included on figure, presented similar sequences. BPV sequence names are labelled as follows: BLAST identification and GenBank accession number. The scale bar represents a genetic distance of 0.1 substitutions per site.

DISCUSSION

The results from this study have demonstrated that all clinical cutaneous lesions that were histologically characterized as sarcoids contained BPV DNA; similar findings were described in North America (Carr et al. 2001), Europe (Angelos et al. 1991, Otten et al. 1993), and more recently by our group in two horses from Southern Brazil (Lunardi et al. 2013b). Investigations to identify the etiologic agent of equine sarcoids have demonstrated that BPV1 and 2 are associated with the pathogenesis of this lesion (Angelos et al. 1991, Otten et al. 1993, Carr et al. 2001, Wobeser et al. 2010). During this investigation, DNA of three BPV types (1, 2, and 13) was amplified from sarcoids. Geographically, BPV1 has been more frequently associated with sarcoids in Europe (Angelos et al. 1991, Otten et al. 1993); while BPV2 is considered as predominant in western Canada (Wobeser et al. 2010). Additionally, the occurrence of the BPV in the USA has been related to BPV2 in Western, and the BPV1 and 2 in Eastern America (Carr et al. 2001). We have recently identified BPV13 DNA in sarcoids from Southern Brazil (Lunardi et al. 2013b), while another research group from Brazil (Silva et al. 2010) has associated the BPV13, previously as designated BPV-BR/UEL4, DNA in a sarcoid from the Southeastern region. These findings suggest that the three bovine *Deltapapillomavirus* might be associated with the development of equine sarcoids in distinct geographical regions of Brazil. Therefore, a detailed epidemiological survey must be done to confirm the possible geographical distribution of BPV types in sarcoids. However, due to the closest relationship between BPV13 and 2, previous diag-

nosis of PV from sarcoids, by restriction fragment length polymorphism analyses (RFLP) (Carr et al. 2001, Wobeser et al. 2010), could have misclassified BPV2 instead of BPV13; therefore we suggest that all PCR products must be sequenced when broad-spectrum primers are used for molecular characterization.

The primer set FAP59/FAP64 resulted in the characterization of the L1 (341 bp fragment) genomic region in 11 sarcoids, while the E5L2fw/E5L2rv pair identified the E5L2 (197 bp fragment) region from 20 sarcoids (Table 2). Moreover, the BPV1 type was more frequently detected relative to BPV2 and 13 during this study. These results are different from a recent study that had identified only BPV13 DNA in sarcoids (Lunardi et al. 2013b). Additionally, the BPV13 DNA was identified in a FFPE sarcoidal sample (Eq.Sar.BRA-11) from 2009 (Table 2) suggesting that this recently described BPV type probably existed before the data of the first report was published. Although the role of BPV13 in the pathogenesis of sarcoids is still uncertain, this BPV type was identified in horses from two regions, South and Southeast Brazil, suggesting that the BPV13 might be widespread in Brazil.

Genomic DNA isolated from archived FFPE tissues is an important tool used in retrospective studies. However, the accuracy of these results can be affected due to DNA degradation and chemical modification of DNA, since these alterations can vary over time or due to prolonged fixation (Hewitt et al. 2008). Consequently, several commercial FFPE kits have been developed to improve the efficiency of DNA extraction. Fragmented DNA extracted from archival FFPE tissue frequently allows for the PCR analysis of only short amplicons that rarely exceeding 300 bp, even with the usage of commercial kits (Jacobs 2012). The difficulty to extract adequate and reliable DNA from FFPE tissue samples might explain the negative results obtained with the FAP59/64 primers, designed to amplify a 480 bp fragment.

Investigations in cattle have demonstrated the occurrence of different BPV types in papillomas from various anatomical locations of the same animal (Claus et al. 2009). During this study, the multiple sarcoids of horse #1 contained only BPV1 DNA. However, the four sarcoids from different anatomical locations of horse # 2 contained the DNA of three distinct BPV types (BPV1, 2, and 13). To the best of the authors' knowledge, this is the first identification of the simultaneous occurrence of three BPV types, from the *Deltapapillomavirus* genus, in the same horse. Moreover, these horses with multiple sarcoids were from the same herd and were reported as in close contact with cattle that were previously diagnosed with cutaneous papillomatosis; this direct contact between cattle and horses might be associated with the diversity of the BPV types identified in horse # 2.

CONCLUSIONS

This study might represent the first description of the simultaneous occurrence of different three BPVs in multiple sarcoids of the same horse and is the first description of BPV1 and 2 in horses from Brazil.

Additionally, due to the high genetic identity among the three BPV types from *Deltapapillomavirus* genus, we recommend sequencing of PCR products when general broad-spectrum primers are used to avoid the misidentification of BPV types DNA in equine sarcoids.

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