



Brief communication

Papillomavirus type 2 in an equine sarcoid in Costa Rica

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ABSTRACT

Objective: To identify, by molecular techniques, the presence of bovine papillomavirus (BPV) in an equine sarcoid and to perform histological characterization. Materials and methods: Using a sarcoid lesion from an American Paint Horse mare, nucleic acid extraction and identification by molecular techniques were performed. A partial region of the gene corresponding to the L1 segment of papillomavirus was amplified by polymerase chain reaction, and then, it was sequenced and analyzed using the BLAST algorithm. The tissue was stained with hematoxylin-eosin and analyzed under a 100X optical microscope. Results: BPV-2 was detected in the equine sarcoid lesion; the lesion was classified as nodular according to its morphological and histological characteristics. The histopathology showed a fibrotic lesion, with exophytic neoplasia and expansion of the spinous layer. **Conclusions:** The study demonstrated, for the first time, the presence of, via molecular techniques, BPV-2 in an equine sarcoid in Costa Rica and its association with a nodular-type sarcoid.

Keywords: Equine; Polymerase chain reaction; Bovine papillomavirus; Fibrosis; Costa Rica (*Source: DeSC*).

RESUMEN

Objetivo: Identificar mediante técnicas moleculares la presencia de papilomavirus bovino (BPV) en un sarcoide equino, y realizar la caracterización histológica de este. **Materiales y métodos:** A partir de lesiones de sarcoide en una yequa de raza pinto americano se realizó la extracción de ácidos nucleicos para su identificación mediante técnicas moleculares. Se amplificó el gen correspondiente al segmento L1 para papilomavirus a través de reacción en cadena de la polimerasa, se secuenció y luego fue analizado utilizando el algoritmo BLAST. Se realizó tinción de hematoxilina-eosina del tejido y se analizó en microscopio de luz 100X. **Resultados:** Se detectó BPV-2 en la lesión de sarcoide equino; el sarcoide se clasificó como nodular de acuerdo con sus características morfológicas e histológicas. La histopatología mostró una lesión fibrótica, con neoplasia exofítica y expansión del estrato espinoso. **Conclusiones:** El estudio demostró por primera vez la presencia de BPV-2 en un sarcoide equino en Costa Rica utilizando técnicas moleculares y su asociación con un sarcoide de tipo nodular.

Palabras clave: Equino, Reacción en cadena de la polimerasa, Papilomavirus bovino, fibrosis, Costa Rica (Fuente: DeSC).

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INTRODUCTION

Equine sarcoid is the most common skin tumor seen in horses around the world. This tumor does not metastasize but can be invasive, generate cosmetic defects and lead to ulcers. If affects the eyelids and can affect vision (1). The development of these lesions in horses is associated with bovine papillomavirus (BPV) infection, although virus infection alone is not enough for tumor development (2,3,4). Sarcoids are classified according to their clinical characteristics as occult, nodular, verrucose, fibroblastic, mixed or malevolent (5,6).

Most studies that employ molecular techniques to determine the presence of BPV in equine sarcoids have been carried out in Europe. Bovine papillomavirus genotype 1 (BPV-1) has been reported in both Europe and Australia. On the American continent, in the United States and Canada, BPV-2 has been identified, and in Brazil, BPV-1, BPV-2 and BPV-13 have been identified (6,7,8). Additionally, Brazil reported the coexistence of the three genotypes in the same equine animal (8).

A study by Vindas et al. (9) in Costa Rica reported the treatment of four horses with a presumptive or histopathological clinical diagnosis of sarcoid. The study found that the two horses that received combined treatment involving surgical removal, cauterization and autovaccine progressed more favorably, without relapsing, than did two other horses that were treated without the administration of the autologous vaccine; further research was recommended (9). The objective of the present study was to identify the presence of bovine papillomavirus (BPV) in equine sarcoids using molecular techniques.

MATERIALS AND METHODS

Sample collection: A 3-year-old American Paint Horse mare, located in Bajo el Remolino, in Buenos Aires de Puntarenas, Costa Rica, presented three sarcoid-type lesions on the left hind limb at the medial level (Figure 1) and one sarcoid-type lesion in the ear; the four lesions had macroscopically identical characteristics. A sample was taken from two lesions on the hind limb, making cuts 1-2 cm in length. One of the sections was preserved in 70% ethanol for molecular analysis (Figure 1A), and the other was preserved in 10% formaldehyde for histopathological analysis until processing (Figure 1B).

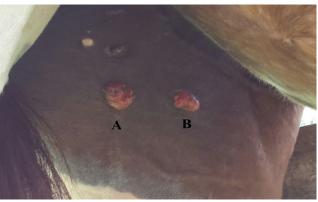


Figure 1. Sarcoid lesions located on the left hind limb of a mare. A. Sample analyzed by PCR B. Sample analyzed histopathologically.

Molecular analysis (DNA extraction, **amplification and sequencing):** The sample preserved in ethanol was subjected to DNA extraction using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Using polymerase chain reaction (PCR), a 478 bp conserved region in the gene encoding structural protein L1 (10) was amplified using the primers FAP59/ FAP64 (5'-TAACWGTIGGICAYCCWTATT-3'/5'-CWATATCWVHCATITCICCATC-3'). PCR was performed with DreamTag Master Mix 1X (Thermo Scientific, USA), 0.25 µM of each primer, 1.25 μ L of DNA (100 ng/ μ L) and nuclease-free water (Thermo Scientific, USA) in a volume of 25 μ L. The conditions for amplification were initial denaturation at 94°C for 10 minutes, followed by 45 cycles of denaturation at 94°C for 90 seconds, hybridization at 50°C for 90 seconds, and extension for 90 seconds at 72°C, with a final extension at 72°C for 5 minutes (11).

Visualization of the PCR product was carried out by electrophoresis using a 1% agarose gel stained with GelRed (Biotium, USA). GeneRuler 1 kb DNA ladder (Thermo Scientific, USA) was used as the molecular weight marker. PCR products were sent for sequencing to Macrogen, Seoul, Korea. Sequence editing was performed with Bioedit 7.2.5 software. Subsequently, the obtained sequence was compared with the sequences in GenBank using the BLASTn algorithm. Using ClustalW[®], alignment of the sequence obtained with reported sequences of 13 existing genotypes was also performed to establish the level of homology of the L1 amplicons.

Morphological and histological analyses:

The sample preserved in formaldehyde was stained with hematoxylin-eosin and analyzed under a 100X optical microscope (12). The lesion was classified based on Knottenbelt (5): occult, verrucose, nodular, fibroblastic, mixed and malevolent (5).

RESULTS

The sequence of the L1 structural protein amplified from the equine sarcoid sample (GenBank accession number MN304951.1) was found to be 100% (400/400 bp) similar to a BPV-2 sequence that had been isolated from a papillomatous lesion on a cow in Japan (GenBank accession number MH589273.1) (Figure 2). The alignment of the obtained sequence with that of the 13 existing genotypes indicated 99% nucleotide identity with BVP-2 (GenBank M20219.1). With the other genotypes, the homology ranged from 49% to 89%.

	10 20 30 40 50 60 70 80	
	10 20 30 40 50 60 70 80 TICTATGIGI TIGTICITIA ATICIAAAGG AGGACAGGA CC-ATITIC TATICIATCA GTAACA CATGGACGGG CTG	
Costa_Rica(MN304951)	TICIATGIGI TIGITCITIA ATICIAAAGG AGGACACGCA CC-ATITIC TATICIATCA GIAACA CAIGGACGGG CIG	
BPV-1 (X02346)	TICIAIGIC IIGITITITA ATICAAGAGG AGGGCAGGCG CCATTITC IAGACGATCA GTAACA CAIGGACGGG CIG TICIAIGIGI IIGITCITIA ATICIAAAGG AGGACACGCA CCATTITC IAGICIAICA GTAACA CAIGGACGGG CIG	
BPV-2 (M20219) BPV-3 (3F486184)	TICIAIGIGI IIGIICITIA ATICIAAAGG AGGACAGCA CC-ATITIC IAGICIAICA GIAACA CAIGGACGG CIA	
BPV-4 (X05817)	TIGTATITIT GIGITITICA GCICAATAGG IGGGCAATCC CC - IGGIC CTIGIG GTIC A CAAGCIIGIG CCI	
BPV-5 (AF457465)	CTCAATAGGC TIGTICITIA ATICIAIGGG AGGACAGICC CCIGCITI IGGCIGITIA CCTICA CAAGGAATAG CII	
BPV-6 (AJ620208)	TTGAATAAGA GTGTTGACTA GCTGTAATGG AGGGCATGCC CC-AATTTC TAATGGTTGG CATGATTCAG CTA	
BPV-7 (DQ217793)	TICAATAAAT GAATTAACTA GTIGTATTGG AGGGCAGIGC TCATCAATAT CAGGATIGIG CGCGICCACA CAGGACTIIG CAA	
BPV-8 (DQ098913)	CTCTATAGGC TIGITCITCA GCTCTATAGG TGGGCAGCCT CC-AGGAGC AGGCCGCTTT TCAGGA CATCIGCAG CTT	
BPV-9 (AB331650)	TIGAIGGIA GAATITACIA GUICIAGIGG AGGACAAICG CC - AGGITI GAGAICIGIG - CAIGCUITIG CAA	
BPV-11 (AB543507)	TICAATTITI GTATICTITA ATICTATIGG AGGACAGICI CCIGGITG AGIGICICGCA GTGGGA CAGGGITIAG CTG	
BPV-12 (JF834523)	TAGTATIGIA GAATICACCA ATTGAATAGG AGGACAGCIG CC-TITIGC TIGIGGITIG CATGGICAG CTI	
BPV-13 (JQ798171)	IICTATGTGT ITGTICTITA ATICTAAAGG AGGACACGCA CC -ATITIC IAGTCTATCA GTAAC -A CATGGACGGG CTG IICTATAATA GTGTITITCA GTCAATAGG GGGACATCGT CC -IGGTC CACCCGGTCA - CATGCTGTG CA CGTGATITI GGTGTITGA GTCCAATAGG GGGCACGC CC - GGTGT CGGCGGTG GTC - A CAAGGATGG CT IIGAATAAGG ITGTICTITA ATICTATGG AGGACAGTCC CC -IGGTT IGGCGGTTA CTC - A CAAGGATGG CT IICAATAAGA GTGTIGACTA GCTGATAGG AGGACAGTCC CC - AGTAC TAAGGTIGG CCCATAAGC ITGTICTICA GCTGTATGG AGGACAGTCC CC - AGTAC TAAGGTIGG CCCATAAGG ITGTIGATGG GTGTATGG AGGACAGTCC CC - AGGAC AGGCCGCTT ICAGG- A CATGCATGG CA CICATAAGG ITGTIGTATGG AGGCCAGCC CC - AGTAC GAGATGTG CGCGCGCCAC AGGACTGC CA CICATAAGG GTGTIGACTA GCTGTATGG AGGCCAGCC CC - AGGAC AGGCCGCTT ICAGG- A CATGCCTGC GA IICAATAAGA GTGTITACAA GCTCAATAG GGGCCAGCC CC - CACATT IGCCTGTG CACGGCCGGC CAAGGCCAGCC CC - AGGAC AGGCCGCTT CCAGG - A CATGCCTGC CA CICATATAGG GTGTITACAA GCTCAATAG GGGCCAAGCC CC -CACATT IGCCTTGTG CCCAATTTI GTATCTTA ATICTATGG AGGACAGCG CC - GCTGGTG GAGTGCTGC - A CAGGGCTGG CTA CICATATTTI GTATCTTA ATICTATGG AGGACAGCGC CC - AGTTGC TAGGTGCGAC GTGGG - A CAGGGTTAG CTA IICAATATTI GTATCTTA ATICTATGG AGGACAGCGC CC - AGTTGC AGGCCGACG GTGGG - A CAGGGTTAG CTA CICATTTTI TGTTCTTA ATICTATGG AGGACAGCGC CC - AGTTGC AGGCCGAC GTGGGC - A CAGGGTTAG CTA CICATATTTI TGTTCTTA ATICTATGG AGGACAGCGC CC - ATTGTC AGCCGGACCA GTGTC - CCTGGTCG CTAGGTCGC CTGGTG CTAGGACGGG CTG IICAATATTI TTGTTCTTA ATICTAAGG AGGACAGCGC CC - ATTGTC AACCCGATCA GTTAC - A CATGGCCGGG CTG	
	110 120 130 140 150 160 170 180 TCTECABGEG TACAGCCCAG CAACAGAATC TGTTGTTGCT TAGCATCTAA TCCTGTTGC TTCCTGTCAT CTGTTGTTG TGC	
Costa Rica(MN304951)	TOTGCAGEGE TACAGECCAE CAACAGAATC TETTETTECT TACATCTAA TOCTETTEC TECTETCAT CTETTETTE TEC	
BPV-1 (X02346)	TCAGCAGGGG TACAGCCTAG CAACAGAATC TGTTGTTGCT TAGCATCTAG GCCTGTTTGT TTCCTGTCAT CTGTTGTTTG GGT	
BPV-2 (M20219)	ICTGCAGGGG TACAGCCCAG CAACAGAATC IGTIGTIGCT IAGCATCTAA ICCTGTITGC IICCIGICAT CIGITGIIIG IGC	
BPV-3 (AF486184)	TCGCAGGGGG TGCAGCCAAC AATAAACATT TGCACCTGCT TAGGGTCTAC AGCCATGTTA ACCCGGTCAT CTGTGGTTTG ATC	
BPV-4 (X05817)	TCACAAGGTA CACAGCCAAC AATAAAAAGC TGAACTIGCT TGGGGTCCAT ACATACATTA ACCCTATCAT CTTCTTTTIG ATC	
BPV-5 (AF45/465)	ATAGCAGGEG TECAGECIAT GAGCAGCATC TGIGTITECT TAACATCCAT TECTAGCIGE TITECTATCAT CTGATCCIEG CAG	
BPV-6 (AJ620208) BPV-7 (DO217793)	TCALAGGEGT TCCAACCAAC ARICAAAAGC IGAACIGCI IIGGAICIAG IGCIGIAIIA ACCCINICAI CIICICCIIG AGA	
BPV-8 (D0098913)	GIGGCIGGIG IACAGCCCAC TAGAAGTATI IGACICIGII IGACATCAAA COCIIGIIGO ITCOIGICAI CIGACCCIIG IGI	
BPV-9 (AB331650)	TCACAAGGAG TGCATCCTAC AATGAAAAGC TGAACCTGCT TAGGGTCCAG ACAAACATTC ACCCTGTCAT CTTTACCTTG GGC	
BPV-10 (AB331651)	TCACAAGGAA TGCAGCCTAC AACAAAAAGC TGTACTTGCT TAGGATCTAC ACACATATTC ATCCTGTCAT CTGTGGTTTG TGT	
BPV-11 (AB543507)	TCACAGGGGAA CACAGCCCAC TATAAACAGC TGCACCTGTT TAGGGTCAAC ACAGACATTG ACCCTGTCAT CTGCATTTTG GGC	
BPV-12 (JF834523)	ICICCAGEGE TACAGECCAE CAACAGARIC TETTETTEGT TAGCATCHA ICCTETTEGT ITCCTETTET ICCTETTETTE TE ICACCAGEGE TACAGECCAE CAACAGARIC TETTETTEGT TAGCATCHA ICCTETTETT ITCCTETAT ICTETTETTE ICICAGEGEGE TACAGECCAE CAACAGARIC TETTETTEGT TAGCATCHA ICCTETTETT ITCCTETAT ICTETTETTE ICICAGEGEGE TACAGECCAE ANITAAACATI TECACIGET TAGGETCAE AGCAGTETA ACCEGETAT ICTETTETTE ICICAGEGEGE ICCAGECAAC ANITAAACATI TECACIGET TAGGETCAE AGCAGTETA ACCEGETAT ICITITITE ATACAGEGET ICCAGECCAE ANITAAACATI TECACIGET TAGACTCAE ICCATACATICAE ICITITITE ICICAGEGEGE ICCAACCAAC ANITAAACATI TECACITICI TAGGETCAE ACAICATITA ACCETATCAT ICITITITE ATACAGEGET ICCAGECCAE ANITAAAAACI TEACTITICTI TAGACTCAE ICCATACATICAE ICITICITITIE ATACAGEGEGE ICCAACCAAC AAITAAAAACI TEACATCAE ITGEGATCAE ICCATACATICAE ICITICITITE ATACAGEGEGE ICCAACCAAC AAITAAAAACI TEACAACATI ITGEGATCAE ICCATACATICAE ICITICITICAE ICI ATACAGEGEGE ICCAACCAAC AAITAAAAACI TEACAACATI ITGEACITAEA ICCATACATICA ICCTETCATICAI ICI ACCAGEGEGA ICCAACCAE IAAIGAAAACI TEACACIGI ITGEGATCAA ACAACATIC ACCCTGACATICAI ICIGACITIGE IE ICACAAGEGA IGCAICCIAC AAIGAAAAGC IGAACCIGI ITGEGAICTAC ACACATACIC ACCCGGICAI ICIGACITIG IE ICACAAGEGA ICCAECCIAC AAIGAAAAGC ICIACITICI IAGGGICCAG ACAAACATIC ACCCIGICAI ICIGACITIG IE ICACAAGEGA ICCAECCIAC AAIGAAAAGC ICIACITICI IAGGAICIAC ACACACATICI ACCCIGICAI ICIGACITIG IE ICACAAGEGA ICCAECCIAC INTAAAAAGA ICICIGICI IAGGAICIAC ACACACATICI ACCCIGICAI ICIGACITIG IE ICACAAGEGA ICAECCICAC AAIGAAAAGC ICICICICI IAGGAICIAC ACACACATICI ACCCIGICAI ICICAITIG IE ICACAAGEGA ICAECCICAC INTAAAAAGA ICICICICI IAGGAICIAC ACACACATICI ACCCIGICAI ICICAITIG IE ICACAAGEGA ICAECCICAC INTAAAAAGA ICICACIGI IE ICICAAGEGA ICAECCICAC INTAAAACAIC ICICAICII IAGGAICIAC ICICAATITI ICICICICIAI ICICAITIG IE ICICAAGEGA ICAECCICAC INTAAAACAIC ICICAICII IAGGAICIAC ICICAATITI ICICICICIAI ICICAITIG ICICAI ICICAAGEGA IACAACCICAC INTAAAACAIC ICICAACAACAIC ICICAATITI ICICICICIAI ICICAICIACIA ICICAITITI ICICICICIAAI ICICAITITI ICICICICIACIAI ICICAITICAI ICICAITICAICAI ICICAAGEGAA IACAACCICACAACAACAIC IEGTTICIICI IACAACIACA ICICAATITI ICI	
BPV-13 (JQ798171)	TCTGCAGGGG TACAGCCCAG CAACAGAATC TGTTGTTGCT TAGCATCTAA TCCTGTTTGC TTCCTGTCAT CTGTTGTTTG GCT	
	210 220 230 240 250 260 270 280	
	environal	
Costa_Rica(MN304951)	CATITICIGC ATCAAGCAGA GCATIAAAAG TGGGGTGCCC AGTAACTGIG CCTCCIAGIG GIIGGCCACG AGACACIIGA ACC	
BPV-1 (X02346)	CATTITICIGE ATCAAGEAAA GEATTAAAAG TEGEGETECCE ACTIACAGTA CETCEAAGAG GETECCECTET GEACACETEC ACA	
BPV-2 (M20219)	CATIFICISC ALCAAGGAGA GCATIAAAAG IGGGIGCCC ANTACISIS CUICTAGIG SIIGGCCACG ACAIACIGA ACC	
BPV-4 (X05817)	GATTITETEC ATCTITERAC TTATRAAC TEGEGEACC TETACTCCT ACACCEACE GETECTCT ACATATITET AT	
BPV-5 (AF457465)	CATTITCIGC ATCTGICCAA ACATIAAACA ATIGATICCC IGIGACAGGG GCICCIAGAG GCIGICCICI ACTIACCIGA AGG	
BPV-6 (AJ620208)	AGTITICIGC ATCTCTAAAT TIGTIGAAAG AAGGGIGGCC AGTAACACCT ATTCCCAAIG GCIGICCCCT GCAGATITCA AIG	
BPV-7 (DQ217793)	GGTTTTCAGT GTCAATC TIGTTAAAAA AGGGATGGCC IGTGCAICCT ACACCTAAIG GGCCICCTCT ACCAATTICA AIG	
BPV-8 (DQ098913)	CATTITCIGC ATCIGICCAA ACATIAAATA AATTAIGGCC IGIGACAGGI GCICCIAAAG GCIGCCCICI ACITACCIGI AGG	
BPV-9 (AB331650)	AATITICIGC AICTITGAAC TIATIGAAAG CAGGETGGCC IGTGACACCT AITCCTAAAG GCIGGCCCCT ACAAATITCA AIG	
BPV-10 (AB331631) BPV-11 (AB543507)	CATIFICAGE ALCEGRAAC CHAIGAALS CAGGAIGACE DESCICE ALACCAAGIG GEGECETET GEALAILTEE ALT	
BPV-12 (JF834523)	TGTTTTCTGC ATCCAGT TTATAAACA AGGGATGCCC TGTAACCCCT ATTCCTAGGG GTTGACCTCT GCAAAATTCT AAG	
BPV-13 (JQ798171)	CATTITICIGE ATCANAGCAGA GCATIABANG TEGGEGECCC AGTAACTEG CCTCCTAGTE GTTEGGCACCA GACACLTICA ACC CATTITICEG ATCANAGCAGA GCATIABANG TEGGEGECCC AGTAACTEG CCTCCTAGTE GTTEGGCACCA GACACLTICA ACC CATTITICIGE ATCANAGCAGA GCATIABANG TEGGEGECCC AGTAACTEG CCTCCTAGTE GTTEGGCACCA GACACLTIGA ACC CATTITICIGE ATCANAGCAGA GCATIABANG TEGGEGECCC AGTACACTE CCTCCAGAGE GTTEGGCACCA GACACLTIGA ACC AGTITICIGE ATCANAGCAGA GCATIABANG AGGEGEGECC AGTACACCAC ATLCCCAAGE GCTEGTCCTC ACCACATITCA ATG CATTITICIGE ATCATICAAL TITGTTABANG AGGEGEGECC CATTACCACAGE GCTEGTCCTC ACTACLCIA AG AGTITICIGE ATCCTTCAAL TITGTTABANG AGGEGEGECC AGTACACCAC ATLCCCAAGE GCTEGTCCTC ACCACATITCA ATG GGTTITICIGE ATCCTCAAL TITGTTABANG AAGGEGAGGCC GATGACACCA TATCCCAAGE GCTEGTCCCT ACCACATITCA ATG GGTTITICIGE ATCCTCAAL TITGTTABANA AGGEGAIGGCC TGTGCACCT ACACACTAGE GCTEGTCCCT ACCACATITCA ATG GATITITICIGE ATCCTCCAAL CATIABANA AATTATGGCC TGTGCACCC ATTCCCAAAGE GCTCCCCCTC ACCACATICA ATG CATITITICAGE ATCCTTCAACC CIATICACAAGE CCTGCCACCAC ATCCCTAAAGE GCTGCCCCCT ACCAATITCA ATG CATITITICAGE ATCCTCAAGE CIATICACAGE CIGGCACCC ATTCCCTAAGE GCTGCCCCCT GCAAATICCT ACC ATTITICAGE ATCCTCAAGE CIATICACAGE CIGGCACCC ATTCCCAAGE GCTGCCCCCT GCAAATICCT ACC ATTITICAGE ATCCTCAAAC CIATICAAGE CIGGGCCCC ATCCCCAAGE GCTGCCCCCT GCAAATICCT ACC ATTITICAGE ATCCTCAAGE CIATICAAGE CIGGCACCC ATCCCCAAGE GCTGCCCCCT GCAAATICCT ACC ATTITICAGE ATCCTCAAGE CIATICAAGE CIGGGGCCC ATCCCCAAGE GCTGCCCCCT GCAAATICCT ACC ATTITICAGE ATCCTCAAGE CIATICAAAG AGGEGAGCCC TGTCAACCAACCCAATICCCAAGE GCTGCCCCCT GCAAATICCT ACC ATTITICAGE ATCCTCAAGE GCATIAAAA AGGGAGGCCC GTAAACCCCT ATCCCAAGGE GTTGCCCCT GCAAATICCT ACC CATITICCGC ATCCAAGE GCATIAAAA AGGGAGGCCC AGTAACCCCT ATCCCAAGGE GTTGCCCCC GCAAATICCT ACC	
	310 320 330 340 350 360 370 380 CAGEGEGETEC ITECITIGEAT INTEGRATE CATTOREAL CONTRACT CATTOREAL CONTRACT CATTOREAL CONTRACT CATTOREAL CONTRACT	
Costa Rica(MN304951)	CAGGCGCTCC ITGCITGGAT IGTGCACAGT CCTATCAGGC AAIGCAAACT GATTGGGATC GGGGAGCTGT AITITAAAAA CTC	
BPV-1(XU2346)	CAGCCGCICI IIACIIGGGI IGIGAACAGI CCIGICAGGI AGIGCAAAII GAIIGGGAIC AGGIAGIIGI AIIIIAAAIA CCC	
BPV-2 (M20219)	CAGGCGCTCC TTGCTTGGAT TGTGCACAGT CCTATCAGGC AATGCAAACT GATTGGGATC GGGGAGCTGT ATTTTAAAAA CTC	
BPV-3 (AF486184)	TAGCCTITGA ITATCAGAGI IGIAIACAII IGGAICGGGA AAACIGAACI IAIIIGGGIC IGGAAACIII AGCCIGAAIA CIC	
BPV-4 (X05817)	TAGTCTTIGE TECTCAGGGT TETATATATE TIGTGTAGGA AGCTAAACT TATTIGGATE AGGCAATTTT AGCTGAAAA CCC	
BPV-6 (AJ620208)	ARGEGERGA GTITICARGAT TEGRANALT TIMICING ANIGUMATI GALINGGIC INGCARCING ANICHARGA CIC	
BPV-7 (DO217793)	CAGCCTATGT GTTICAGGAT TATAAAGTT IGGGTCTGCA AACACAAATT IGTTAGGGTC IGGCAGTTIG ATTCIGATG CTC	
BPV-8 (DQ098913)	CAGGCGCTCC ITGCITGGAT IGTGCACAGI OCTATCAGGC AATGCAAACI GATTGGGATC GGGAGCCGI ATTTTAAAAA CIC IAGCCTTGA ITATCAGAGI IGTATACATI TGGAICGGGA AAACTGAACI IATTGGGATC GGGAGACITI AGCCTGAAAT CIC IAGCCTTGA ITATCAGAGI IGTATACATI TGGAICGGA AAGCTAAACI IATTGGGATC AGGGAGATTI AGCCTGAAAA CIC CAATGTTTGC ITTCCAGGGT IATGTAAATI TITATGTGGC AGTGCAAATI GATTAGGGTC IGGCAGATGI AGCCGAAAA AGGCGCGAG GTTICAGGAT IATGTAAATI IGTGGITGG AAAGCTAAACI IGTTAGGGTC IGGCAGTGA AGCTAAAACA CIC CAGCCTAIGI GTTICAGGAT IATGAAATI IGGGITGGC AAGCAAAATI IGTIAGGGTC IGGCAGTTG ATCTGAAAG CIC CAGCCTAIGI GTTICAGGAT IATGAAATG ITGGCGTCGC AACCACAATI IGTIAGGGTC IGGCAGTTG ATCTGAAAG CIC CAGCCTAIGI GTTICAGGAT IATGAAATG ITGCCGGCAA AGGCAAATI GATTGGGTC IGGAAGCTTA ACCTGAAACA CIGCCTGGCGTI IGTAIAACI TGGGTTGCA AACCACAATI IGTITGGGTC IGGAAACITA ACCCTAAACA CAGCCGTIGC ITGCCAGGGT CATAAAATG ITGCCGGGA AGGCAAATI IGTITGGGTC IGGAAACITA ACCCTAAACA CAGCCGTIGC ITGCIAGGGT CATAACAATI GGGTGATIGA AACCTGAACI IGTITGGGTC IGGAAACITA ACCCGGAAC CAGCCTIGA ITTICAGGGT CATAACACII GGGTGATIGA AACCGAAACI IGTITGGGGTC IGGAAATICA ACCCGGAAC CIC TAGCCTGGAG ITTICAGGAT ATAAACATI IGGATIGGA AACCGAAACI IGTITGGGTC IGGAAATICA ACCCGGAAC CIC AGGCCGTIGC ITGCCAGGAT CATAACACII GGGTGATIGA AACCTAAACI ACCTI CAGCCTGGAT ITTICAGGAT IGTAACACII GGGTGATIGA AACCTAAACI IGTITGGGTC IGGAAATICA ACCCGGAACA CIC AGGCCGCCC ITGCTGGAT ITATAACACII TGGATIGGA AACCTAAACI GATAGGGTC AGGAATATIC ACCCGGAACACI CAGGCGCCCC ITGCTGGAT IGTGAACCII TITACAGGA AACTAAACI GATAGGATC AGGTAAACI CACTAAAAA CIC AAGCCGCCCC ITGCTGGAT IGTGAACTGI TITACAGGC AACGAAACI GATAGGATC AGGTAAACI CACTAAAAA CIC	
BPV-9 (AB331650)	TAGTCTTTGA CTGTCAGGAT TGTATACATC TGGATTCTGG AAGCTAAATT TGTTAGGGTC TGGAAACTTT AGCCTAAAGA CCC	
BPV-10 (AB331651)	AAGCCGTTGC TTGTCAGGGT CATATACATT GGCATTTGGA AAGCTGAACT TGTTGGGGTC TGGAAATTTG AGTCTGAACA CTC	
BPV-11 (AB543507)	AAGTCTITGA TITICAGGGT TGTACACATI GGGTGAITGA AAGCTAAACT TGTITGGATC TGGAAAATITC ATCCGGAACA CTC	
BPV-12 (JF834523) BPV-13 (J0798171)	TAGUCTUGA TUCUUGAT TATAAACATI IGGATTAGGA AAACTAAATI TUTTAGGUU AGGAATAUC AGUCTAAAAA CUC	
Brv-13 (00/301/1)	AND COLORING TO CARDON CONTRACT CATAGORIE AND	
	410 420 430 440	
	energieses [energieses] energieses] energieses] energieses]	
	ACTITIGGAA CAGTITT GTCCCCC ACAGIGACIT GGT	
BPV-1 (XU2346) BPV-2 (M20210)	ACCITAGGAA CAGITIT GGCCCCG ATAGACACIG GGT	
BPV-3 (AF486184)	ACTITEGERA CCACACCTI GICCICA CETITATET CA	
BPV-4 (X05817)	ACTITIGGGAA CAACAACGGT GTCTCCT TTTTTTAGGT CAA	
BPV-5 (AF457465)	ACTITIGGAA TATTCT GTTC TGGAATIT TCC	
BPV-6 (AJ620208)	ACCIIGGGCA CAAIGACIIC CICICCI TITITGAGCI CAA	
BPV-7 (DQ217793)	ACCTFGGGAA CIACIAITITI AGTICITICO CARTIGIAAA CIG	
BPV-8 (DU098913) BPV-9 (AB331650)	ACTITISSEAR IRIIII GAICREELAEGI IGA	
BPV-10 (AB331651)	ACTITIGGGCA CCAATATITT TCCTTCCAAG TCCTTGATGT CAA	
BPV-11 (AB543507)	ACCTTAGGAR CAGTITI GGCCCCG ATAGACACTG GGT ACTTIGGGAR CAGTITI GGCCCCC ACAGCACTT GGT ACTTIGGGAR CAGCAACGGT GTCCCCA CGTTAATGT CAR ACTTIGGGAR CARCAACGGT GTCCCCT TITITTAGGT CAR ACTTIGGGAR TATICT GTCGGARTIT TCC ACCTGGGAC CARACACTC CICTCCT TITITCAGCT CAR ACCTGGGAR CTACTATTR AGTTCTTCC TCATGTTAAR CTG ACCTGGGAR TATITI GATC AGGCAGGT TCA ACCTTGGGAR TATITI GATC AGGCAGGT TCA ACTTIGGGAC CCACATCTC GTGCCCTTGTTAAGGT CAR ACTTIGGGCA CCACATCTTI TCCTTCCAAG TCCTTCATGT CAR ACTTIGGCA CCACATCTTI TCCTTCCAAG TCCTTCATGT CAR ACTTIGGCA CCACATCTTI TCCTTCCAAG TCCTTCATGT CAR ACTTIGGCA CCACTGTTTT GCCCCCTTTTTCCAGGT CAR	
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Figure 2. Sequence alignment of the L1 gene in a sarcoid on a horse in Costa Rica and 13 existing bovine papillomavirus genotypes. Identical regions are shown in gray.

The sarcoid was classified as nodular type A based on its morphological and histological characteristics. Microscopic analysis indicated a fibroblastic lesion. Histopathology indicated an exophytic neoplasia and expansion of the spinous layer in some portions of the lesion (Figure 3).

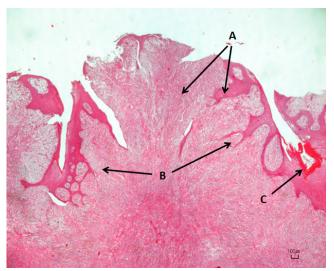


Figure 3. Histopathology of an equine sarcoid on a mare's limb. Hematoxylin and eosin staining revealed the following: (A) exophytic neoplasm composed of epidermal and mesenchymal neoplastic cells, (B) epithelial extensions and (C) expansion of the spiny layer via hyperkeratosis

DISCUSSION

The identification of BPV-2 in an equine sarcoid in Costa Rica is consistent with results from investigations in the USA and Brazil and represents the first report of BPV-2 in an equine sarcoid in Costa Rica and Central America (8). The presence of the virus in the sarcoids of horses can explain the results obtained by Vindas et al. (9), who found relapses in horses that did not receive an autologous vaccine, while two other animals that did receive an autovaccine were relapse free (9).

The lesion was determined to be fibroblastic, as determined by microscopy, because substantial fibrosis was present. The fibroblastic lesions that were observed are consistent with the description by Knottenbelt (5), i.e., a fleshy appearance, with the groin, eyelids, and inner part of the extremities as predominant locations (5). The histopathological findings indicated a exophytic neoplasia, with epidermal cells organized in epithelial extensions, known as rete pegs, projecting towards the underlying connective tissue of the skin and expansion of the spiny layer in some areas of the lesion, findings that are characteristic of sarcoids (13,14). According to Chambers et al. (4), fibroblast infection and the induction of fibroepithelial tumors are attributable to BPV-2 (4). Currently, no study has reported an association between a specific BPV genotype and the classification established by Knottenbelt (5) or between genotype and the sites where lesions manifest in equines.

Although BPV infection is necessary for the development of a sarcoid, the mechanism of equine sarcoid development is unknown (15). It is believed that genetic predisposition and trauma can play important roles in the development of lesions because the presence of BPV has been demonstrated in healthy horse skin (4,15,16). Few studies exist on equine sarcoids and are generally limited to case reports. We recommend conducting larger studies to determine the percentage of equine sarcoids with viral etiology and the genotypes present in these lesions to better assess the use of autovaccines or homologous vaccines as treatment options.

In conclusion, the presence of BPV-2 was identified for the first time in an equine sarcoid in Costa Rica. Further studies are recommended to determine the presence of other BPVs associated with equine sarcoids and the percentage of BPV involvement in equine sarcoids in Costa Rica.

Conflict of Interest

There are no conflicts of interest related to this study, including the preparation of the manuscript and the publication of the article.

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