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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 yegua 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).

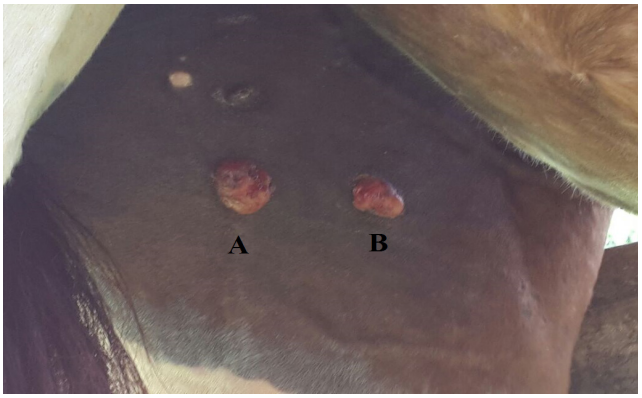

Figure 1. Sarcoid lesions located on the left hind limb of a mare. A. Sample analyzed by PCR B. Sample analyzed histopathologically.

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 DreamTaq Master Mix 1X (Thermo Scientific, USA), 0.25 µM of each primer, 1.25 µL of DNA (100 ng/µL) and nuclease-free water (Thermo Scientific, USA) in a volume of 25 µL. 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%.

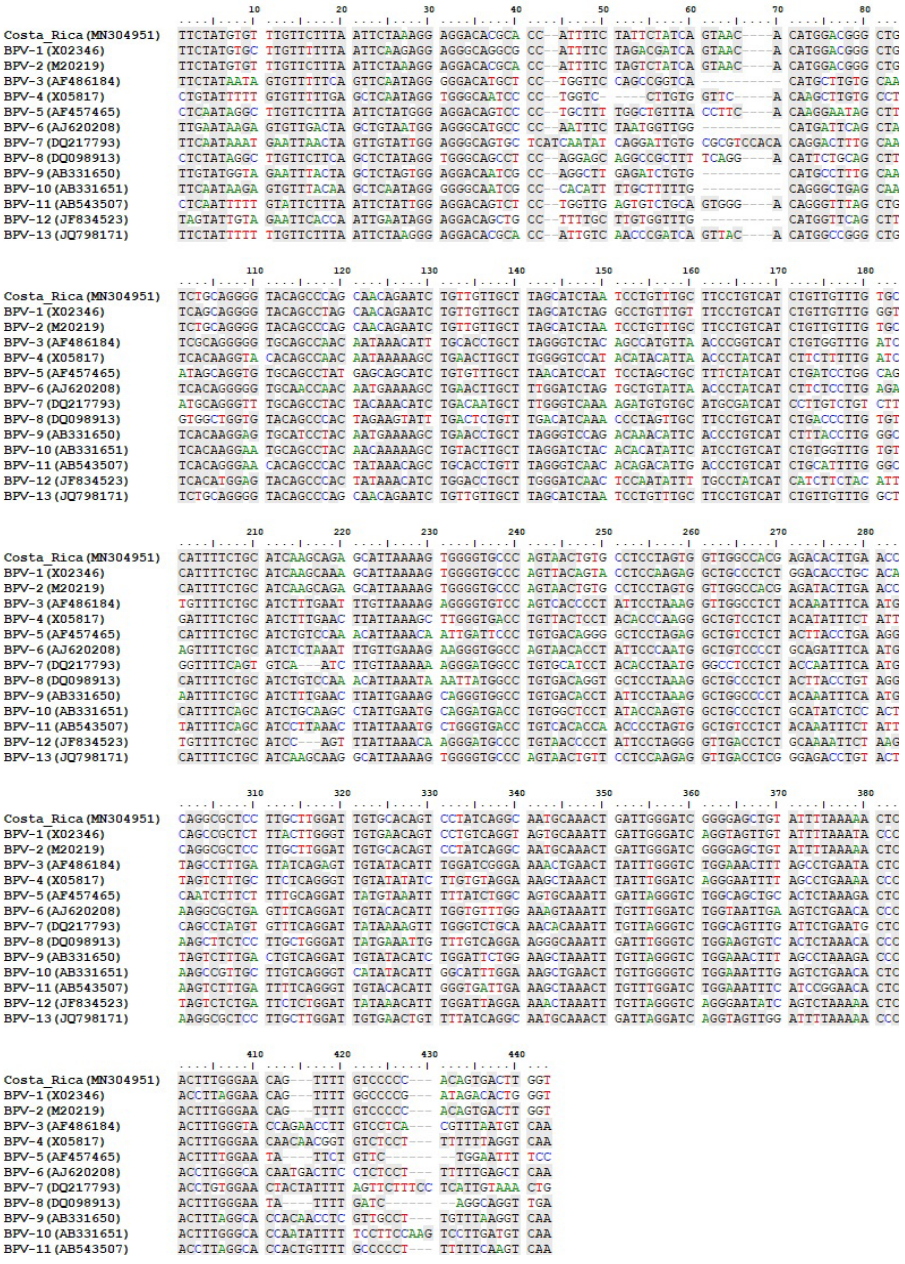

Figure 2. Sequence alignment of the L1 gene in a sarcoid on a horse in Costa Rica and 13 existing bovine papillomavirus genotypes. Identical regions are shown in gray.

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).

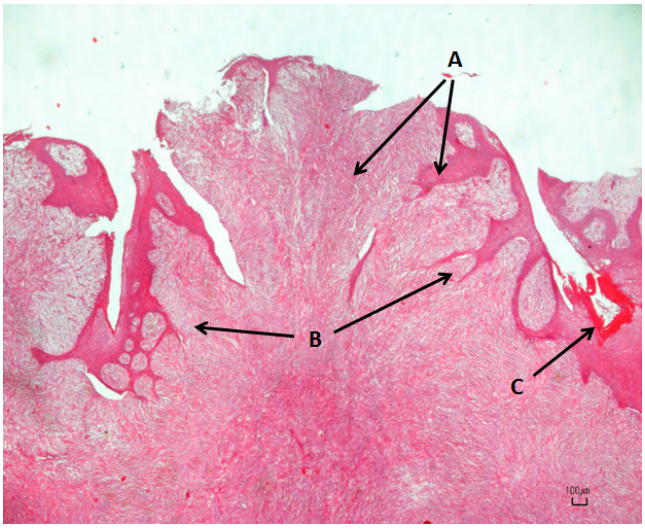

Figure 3. Histopathology of an equine sarcoid on a mare's limb. Hematoxylin and eosin staining revealed the following: (A) exophytic neoplasm composed of epidermal and mesenchymal neoplastic cells, (B) epithelial extensions and (C) expansion of the spiny layer via hyperkeratosis

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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