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DETECTION AND MOLECULAR CHARACTERIZATION OF BOVINE PAPILLOMAVIRUS SUBTYPES IN CATTLE FROM TWO COLOMBIAN REGIONS

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Abstract: Bovine papillomaviruses (BPV) are oncogenic viruses developing benign and malignant hyperplastic lesions of cutaneous, mucosal, upper gastrointestinal epithelia, and bladder in cattle. In this study, we report the subtypes of BPV isolated from Colombian cattle and their molecular characterization. Sixteen biopsies were sampled from papillomatous lesions in two Colombian cattle farming regions. Detection and subtype identification were done by amplifying a 450 bp fragment of the L1 gene using the consensus primers MY09/MY11, followed by Sanger sequencing. Fourteen out of 16 samples tested positive for BPV. Twelve of these 14 samples were clustering with the BPV2 subtype and two with BPV3. The BPV phylogeny analysis showed that the BPV2 Colombian isolated clusters according to the geographical areas of origin. Furthermore, the L1 amino acid substitution most often found in the fragment analyzed in the BPV-2 Colombian isolates was leucine to valine at 386 (Leu386Val). As far as we know, this is Colombia's first report of BPV subtypes; BPV2 was the most common strain found, and BPV1 was not identified.
Keywords: Bovine papillomavirus (BPV), BPV2, BPV3, Colombia, papillomatous lesions.

INTRODUCTION

Bovine papillomaviruses (BPV) are oncogenic viruses that could develop benign and malignant hyperplastic lesions of cutaneous and mucosal epithelia in cattle. In addition, they have been found to act synergistically with bracken fern poisoning in developing bladder and upper gastrointestinal carcinoma in these animals (Daudt et al., 2018). The BPV virion is a non-enveloped icosahedral structure of 55-60 nm diameter, containing a double-stranded circular DNA. The viral genome is composed of three different regions: the long control region (LCR) containing the elements necessary for replication and transcription of the viral DNA, and two regions corresponding to early (E1-E7) and late (L1-L2) genes (Vázquez et al., 2012).

Homology in the L1 gene is used for the phylogenetic classification of papillomaviruses (PV) because it is the most conserved gene within the *Papillomaviridae* family. It thus facilitates further comparisons between PV using partial gene sequences. Differences in >10% in DNA sequence identity are long accepted as a cut-off level to discriminate between different strains or subtypes of PV (De Villiers et al., 2004; Bernard et al., 2010). Papillomaviruses have been isolated in over 20 species of mammals and reptiles (Doorbar, 2005). Twenty-eight BPV types, distributed in five genera, have been characterized and associated with different histopathological lesions (Russo et al., 2020; PaVE, 2021).

Currently, no reports of BPV strains from Colombia have been reported. Therefore, this study presents the first subtype identification and molecular characterization of bovine papillomavirus strains in Colombian cattle.

MATERIALS AND METHODS

Sixteen biopsies were obtained from papillomatous lesions in two Colombian cattle farming regions: six from La Cumbre region-Valle Del Cauca (COL_VC) (3038'59"N 76034'6"W) and ten from Juan de Acosta region-Atlántico (COL_A) (10049'43"N 7502'6"W) (Figure 1). The biopsy was collected through minor surgery by a veterinarian. Biopsies were submerged in PBS 1X and stored at -20°C until their processing. The Institutional Committee from Universidad del Valle approved the protocol for Ethical Review with Animals in Biomedical Experimentation, ID: 001-016.

The DNA was extracted using the DNeasy kit following the manufacturer's protocol. BPV DNA was detected through partial amplification of the L1 gene (450 bp) using the consensus primers MY11; 5'- CGT CCM ARR GGA WAC TGA TC-3' and MY09; 5´-CGT CCM ARR GGA WAC TGA TC-3'. PCR was made in a final volume of 20 µL containing 100 ng/µL of extracted DNA. The PCR conditions were adapted and standardized from another study (Ata et al., 2018): initial denaturation at 95 °C for 5 min, followed by 40 cycles at 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, with a final extension of 10 min at 72 °C. Amplicons were visualized by 2% agarose gel electrophoresis.

BPV subtype identification was carried out in the BPV-positive samples through Sanger sequencing of the 450 bp amplified region of the L1 gene. The sequences were aligned with the BPV L1 gene reference sequences using the BLASTn algorithm from the NCBI. BPV subtypes used as references are listed as follows: NC 001522.1 (BPV1); M20219.1 (BPV2); AF486184.1 (BPV3); X05817.1 (BPV4); AF457465.1 (BPV5); AB845589.1 (BPV6); DQ217793.1 (BPV7); DQ098917.1 (BPV8); AB331650.1 (BPV9); AB331651.1 (BPV10); AB543507.1 (BPV11); JF834524.1 (BPV12); JQ798171.1 (BPV13); KR868228.1 (BPV14). BPV phylogeny was performed using the available methods in the phylogenetic inference program MEGA6 (Tamura et al., 2013). Reference L1 region sequences were converted to FASTA format and aligned with the sequences obtained from samples using the Clustal W algorithm (Thompson et al., 1994). The model of DNA substitution for this region was selected according to the lowest value of the Bayesian Information Criterion as implemented in the Model program (Goldman, 1993). The Maximum likelihood method with Gamma distribution with five categories plus invariant sites, with 1000 bootstrap replicates, was used to build the phylogenetic tree, assuming clusters with bootstrap values greater than 75% had a high level of nodal support.

RESULTS AND DISCUSSION

Fourteen out of 16 samples tested positive for BPV. Nine out of 10 samples from the Juan de Acosta region (Atlántico, COL_A) were positive and had a sequence identity greater than or equal to 94% with the BPV2 subtype. Five out of 6 samples from the La Cumbre region (Valle del Cauca, COL_VC) were positive for BPV. Three of these five positive samples showed 99% identity with BPV2 subtypes, and the two others showed identity higher than 97% with BPV3 subtypes. The phylogeny of L1 DNA sequences showed high resolution of most internal nodes with bootstrap value >75%, including a well-supported clade for samples COL_VC3, _VC4, and _VC5 with BPV2 and COL_VC1 and VC_2 with BPV3 (Figure 2). According to MEGA, the Tamura 3-parameter plus gamma distribution plus invariable site (T92 + G + I) was the most appropriate nucleotide substitution model.

For the 12 BPV2-positive samples, 133 variable sites were identified in the L1 gene. Most variable sites, 90 (67.7%), were synonymous, and 43 (32.3%) were non-synonymous substitutions. Furthermore, 64 (43.2%) substitutions were transitions, and 84 (56.8%) were transversions. BVP2-positive samples from Valle del Cauca showed one or two non-synonymous substitutions. In the BPV2-positive samples from Atlántico, the non-synonymous substitutions vary from one substitution in COL_A3 and _A9 to 16 in COL_A8. The most common L1 amino acid substitutions, translated from the region amplified and sequenced, were: Leu386Val, Lys442Arg, and Leu456IIe (Table 1). The non-synonymous substitutions Arg345Lys

and Asp354Ala were identified in the BPV-3 isolates from Valle del Cauca.

Fourteen out of 16 samples of papillomatous lesions from two geographically distant livestock farms in Colombia were positive for BPV2 (12/14) and BPV3 (2/14). The latter is restricted to southern Colombia (La cumbre region), while the former is this study's most common BPV subtype (Figure 2). In South America, Brazil is the country with the most BPV reports. BPV2 has been detected in *Bos primigenius taurus* and equine epithelium in this country, associated with equine sarcoidosis (Otten et al., 1993; Vázquez et al., 2012; Alcântara et al., 2015). BPV2 has also been discovered in the womb, amniotic fluid, and placenta and is described as a causal or inductor agent of abortions or fetal abnormalities. It has also been identified in commercially frozen sperm cells, which has generated concerns about Brazil by the extensive use of frozen semen in the artificial insemination industry; in cutaneous papillomas, fibropapillomas, and even urinary bladder tumors (Roperto et al., 2008; Vázquez et al., 2012). More recently, a study demonstrated that BPV2 infects epithelial cells of the amnios and induces the development of papillomavirus-associated amniotic papillomas in water Buffaloes (Russo et al., 2020). VPB3 has only been identified in cutaneous papillomas and healthy skin of cattle (Vázquez et al., 2012; Bertagnolli et al., 2020).

BPV2 shows a broad distribution worldwide. This subtype has been detected in *Bos primigenius taurus* in Germany, Brazil, New Zealand, Japan, India, Italy, Turkey, and South Korea. It has also been detected in equine sarcoid in the United States, United Kingdom, Poland, Switzerland, and Australia (Vázquez et al., 2012). In contrast, the BPV3 distribution is more limited, having been detected in bovine cutaneous papilloma and healthy skin in Germany, Japan, Brazil, and China (Vázquez et al., 2012).

Although the actual prevalence of BPV subtypes is not known, BPV1 and BPV2 are the most frequent subtypes found (Vázquez et al., 2012). Most of our samples were BPV2-positive, which differs from a recent study in Egypt in which all the 123 cutaneous warts samples analyzed were BPV1-positive (Ata et al., 2018). In Brazil, another South American country, BPV1 was the subtype most frequently identified in cutaneous warts samples (51%, 16/31) and BPV2 the second one (29%, 9/31). Interestingly, BPV3 was not identified (Bertagnolli et al., 2019), but there are reports of this subtype in Brazil (Vázquez et al., 2012). The differences in detection frequency of BPV subtypes suggest different subtypes compared with other countries; however, it is impossible to confirm this hypothesis due to the low sample number and regions analyzed. These differences also may reflect different uses of detection and identification primers or anatomical sampling sites.

Here we report the detection of BPV2 and BPV3 in two farming regions in Colombia. As far as we know, this is the first study reporting subtypes of BPV in Colombia and contributes to expanding this virus's knowledge in the country. However, more molecular studies are needed to identify the BPV subtypes present in other Colombian farming regions to know the prevalence of this virus and what strains are circulating, thus improving the tools for controlling or eradicating BPV-associated pathologies.

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CONFLICT OF INTEREST

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the paper's content.

REFERENCES

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, 431-436. http://dx.doi.org/10.1590/S0100-736X2015000500007

Ata, E.B., Mahmoud, M.A.E., Madboli, A.A. (2018). Molecular detection and immunopathological examination of Deltapapillomavirus 4 in skin and udder of Egyptian cattle. *Veterinary World*, 11, 1-6. https://doi.org/10.14202/vetworld.2018.915-920

Bernard, H.U., Burk, R.D., Chen, Z., van Doorslaer, K., zur Hausen, H., De Villiers, E.M. (2010). Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology*, 401, 70-79. https://doi.org/10.1016/j.virol.2010.02.002

Bertagnolli, A.C., Bezerra, A.V., Santos, R.N., Cavalli, L.S., Varela, A.P.M., Reis, E.M., Cibulsky,S.P., Roehe, P.M., Mayer, F.Q. (2020). Clinicopathological characteristics and papillomavirus types in cutaneous warts in bovine. *Brazilian Journal of Microbiology*, 51, 395-401. https://doi.org/10.1007/s42770-019-00121-2

Daudt, C., Da Silva, F.R.C., Lunardi, M., Alves, C.B.D.T., Weber, M.N., Cibulski, S.P., et al. (2018). Papillomaviruses in ruminants: An update. *Transbound and Emergind Diseases*, 65, 1–15. https://doi.org/10.1111/tbed.12868

De Villiers, E.M., Fauquet, C., Broker, T.R., Bernard, H.U., zur Hausen, H. (2004). Classification of papillomaviruses. Virology, 324, 17-27. https://doi.org/10.1016/j.virol.2004.03.033

Doorbar, J. (2005). The papillomavirus life cycle. *Journal of Clinical Virology*, 32, 7-15. https://doi.org/10.1016/j.jcv.2004.12.006
Goldman, N. (1993). Statistical tests of models of DNA substitution. *Journal of Molecular Evolution*, 36, 182-198. https://doi.org/10.1007/BF00166252

Otten, N., von Tscharner, C., Lazary, S., Antczak, D.F., Gerber, H. (1993). DNA of bovine papillomavirus type 1 and 2 in equine sarcoids: PCR detection and direct sequencing. *Archives of Virology*, 132, 121-131. https://doi.org/10.1007/BF01309847
PaVE (2021, May 22). PapillomaVirus Episteme. Retrieved from https://pave.niaid.nih.gov/

Roperto, S., Brun, R., Paolini, F., Urraro, C., Russo, V., Borzacchiello, G., Pagnini, U., Raso, C.,

Rizzo, C., Roperto, F. (2008). Detection of bovine papillomavirus type 2 in the peripheral blood of cattle with urinary bladder tumours: possible biological role. *Journal of General Virology*, 89, 3027-3033. https://doi.org/10.1099/vir.0.2008/004457-0

Russo, V., Roperto, F., De Biase, D., Cerino, P., Urraro, C., Munday, J.S., et al. (2020). Bovine Papillomavirus Type 2 Infection Associated with Papillomatosis of the Amniotic Membrane in Water Buffaloes (Bubalus bubalis). *Pathogens*, 9, 1–15. https://doi.org/10.3390/pathogens9040262

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725-2729. https://doi.org/10.1093/molbev/mst197

Thompson, J.D., Higgins, D.G., Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. *Nucleic Acids Research*, 22, 4673-4680. https://doi.org/10.1093/nar/22.22.4673

Vázquez, R., Duch, C.E., Gómez, A.D., Duato, E.G., Rico, L.B. (2012). Papilomatosis bovina: epidemiología y diversidad de papilomavirus bovinos (BPV). *Revista Complutense de Ciencias Veterinarias*, 6, 38-58. http://dx.doi.org/10.5209/rev_RCCV.2012.v6.n2.41086

TABLE LEGEND

Amino acid change	Col VC			Col A									
	3	4	5	1	2	3	4	5	6	7	8	9	TOTAL A
Thr**329**Cys					▪								1
Asp**332**Tyr					▪								1
Asn**333**Asp							▪						1
Gly**336**Val							▪						1
Gly**336**Ile								▪	▪				2
Asn**338**His							▪						1
Leu**339**Phe										▪			1
Ser**342**Arg										▪			1
Asn**348**Ser							▪						1
Ala**349**Glu				▪									1
Ala**349**Thr										▪			1
Glu**366**Asp											▪		1
Lys**369**Arg											▪		1
Leu**370**Arg											▪		1
Ser**378**Cys											▪		1
Leu**386**Val		▪		▪	▪	▪	▪	▪	▪	▪		▪	9
Leu**386**Gly											▪		1
Ser**387**Gly											▪		1
His**388**Cys											▪		1
Leu**389**Val											▪		1
Met**393**Gln											▪		1
Asn**399**Asp											▪		1
Glu**401**Gln											▪		1
Ser**409**Pro											▪		1
Ser**410**Pro											▪		1
Ile**419**Leu											▪		1
Gly**440**Ala											▪		1
Lys**442**Arg	▪	▪	▪										3
Leu**456**Ile				▪	▪				▪				3
Pro**460**Ala											▪		1
TOTAL B	**1**	**2**	**1**	**3**	**4**	**1**	**5**	**2**	**3**	**4**	**16**	**1**	

Table 1. BPV-2 non-synonymous substitution mutations in the partial L1 gene sequence. COL VC: samples from La Cumbre-Valle del Cauca. COL A: samples were collected in Juan de Acosta- Atlántico. TOTAL A: the number of non-synonymous changes per position within the L1 sequence. TOTAL B: the number of non-synonymous amino acids changes per sample. In red, the most common amino acid substitution

FIGURE LEGEND

Fig. 1. Morphology of a specimen of cattle collected in two Colombian cattle farming regions corresponding to La Cumbre region-Valle Del Cauca, called "Hartón del Valle" and to Juan de Acosta region-Atlántico, called "Costeño con Cuernos."

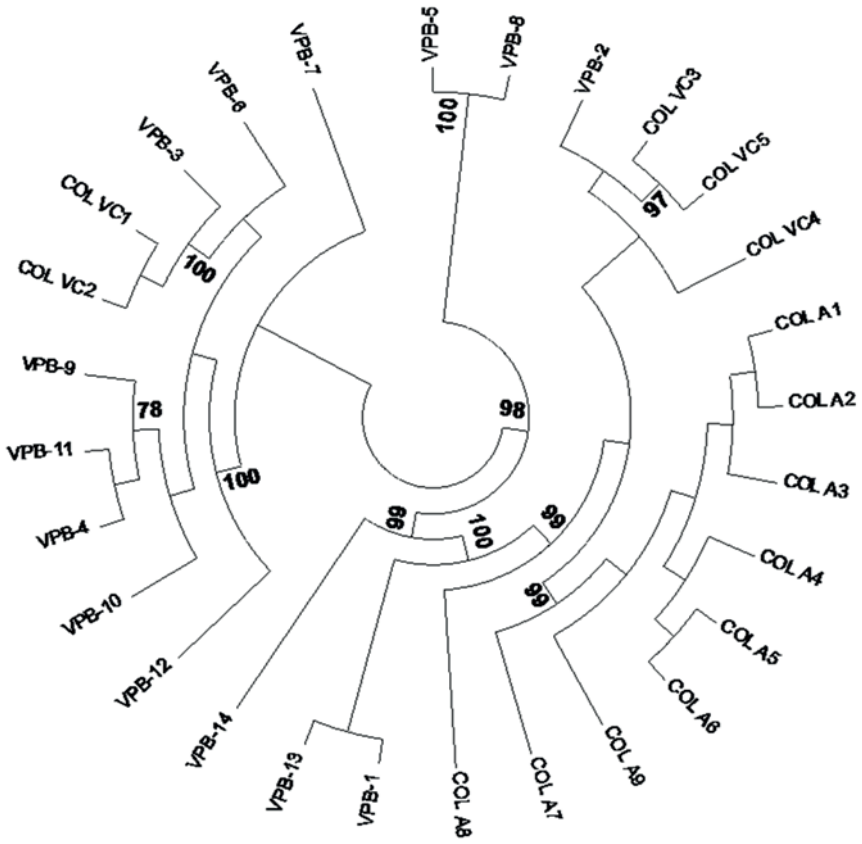

Fig. 2. Phylogenetic tree inferred based on L1 gene with Maximum likelihood (ML) with the Tamura 3-parameter plus gamma distribution plus invariable site (T92+G+I) model. Numbers on branches indicate the bootstrap support values. BPV Colombian isolates from La Cumbre-Valle del Cauca (COL VC) and Juan de Acosta-Atlántico (COL A).